

SOME PHYSICAL PROPERTIES OF GELATIN FILMS
IN RELATION TO HARD CAPSULE PRODUCTION

by

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This book is respectfully dedicated to the many people who
were my colleagues and friends at Nottingham University
1979 - 1983.

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ABSTRACT

Hard gelatin capsules are manufactured from blends of limed ossein (LOG), acid ossein and acid pigskin gelatins, with LOGS usually forming the main portion of the blend. **Unfortunately**, the quality of the finished capsule cannot be predicted by routine quality control tests and 'poor' batches of LOG are encountered which form unsatisfactory capsules. In addition, differences between gelatin types are encountered in practice but the supporting literature is sparse and conflicting.

This work examines some properties of gelatins pertinent to hard capsule manufacture in an attempt to relate these to gelatin type and known performance on capsule machines.

Molecular mass distributions (MMD) were examined by polyacrylamide gel electrophoresis. Each different gelatin type possessed a characteristic shape of MMD which could be related to the source tissue and the treatment undergone during gelatin manufacture. MMDs, isoionic point determinations and an assessment of the content of protein impurities by ultraviolet spectrophotometry failed to resolve differences between 'good' and 'poor' LOG batches.

The drying rates of freshly-cast gelatin films were studied under conditions of controlled humidity, temperature and air velocity. No significant differences between gelatin types was observed. Small variations in equilibrium moisture content were seen and were tentatively ascribed to the generation of water binding sites (free carboxyl groups) during gelatin manufacture.

The mechanical properties of dry gelatin films were examined by tensile stress-strain and stress-relaxation measurements. Viscoelastic behaviour typical of a polymer in the glass transition region was observed, and significant differences in film fracture strain were observed but these were not related to gelatin type. Optical rotation measurements indicated similar orders of film crystallinity and determinations of frictional characteristics similarly revealed no varietal differences.

Overall, few differences related to gelatin type were seen within the properties examined. There was no evidence in these studies to explain the unsatisfactory behaviour of 'poor' LOG batches in capsule manufacture.

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CHAPTER I

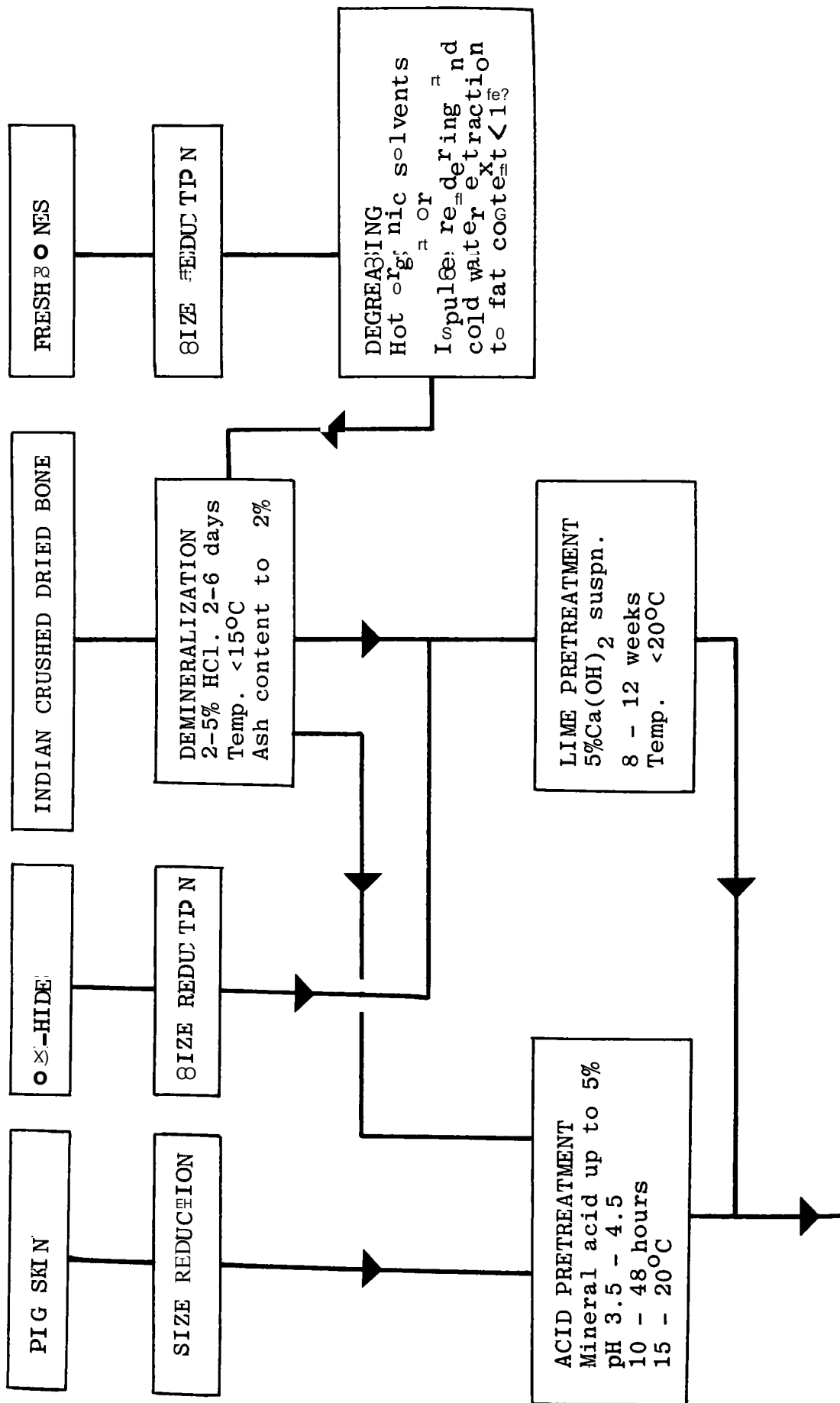
INTRODUCTION

1.1 GELATIN MANUFACTURE

Pharmaceutical grade gelatins suitable for hard capsule production are manufactured from the collagenous component of animal skins and bones. The raw materials are crushed dried bones from India, fresh bones from the slaughterhouse, ox-hide from tannery wastes and (particularly in North America) pigskins,

A flow diagram of the main stages of gelatin manufacture is shown in Figure 1". There are many variations. Good reviews are provided in references 3 to 7. Pigskins, because of their high **fat content**, are almost invariably acid pretreated, ox hides are limed, and ossein is suitable for either (7). The '**pretreatment**' stage is a controlled hydrolytic degradation of the native collagen that greatly improves the yield, extraction rate and physical properties of the gelatin subsequently extracted (1).

Extraction at first employs relatively low temperatures in which gelatin molecules are solubilized by hydrogen bond rupture, a process termed 'thermal shrinkage'. This is aided by the limited scission of peptide bonds and covalent crosslinks that has occurred during pretreatment (10). Later extractions employ increasingly higher temperatures and both higher and lower molecular mass species are liberated by further covalent bond breakage.



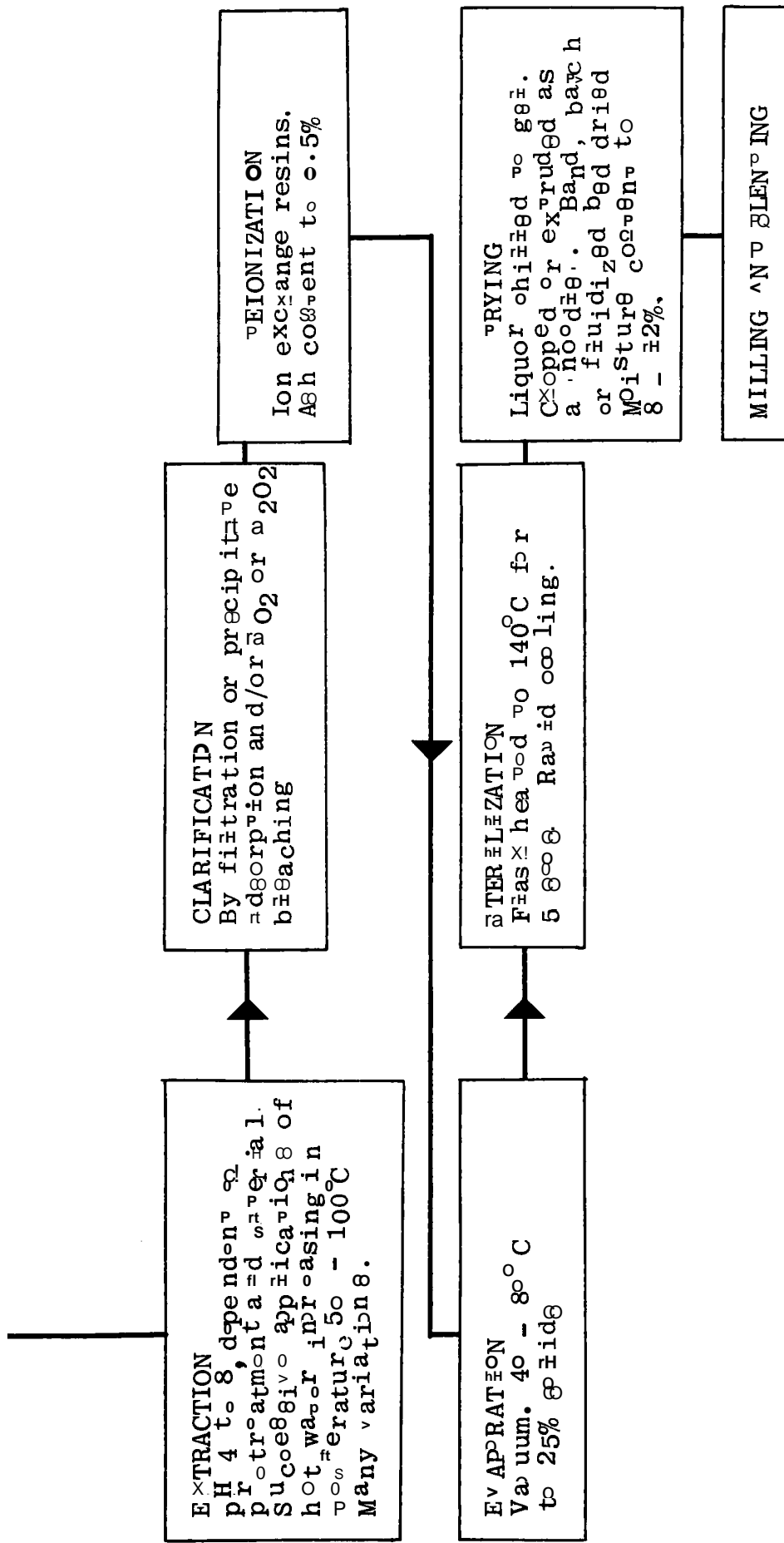


Figure 1.1
The Manufacture of Gola in
(After 1900-1908 2-7)

The broadening of the molecular mass distribution is manifest in poorer physical properties of successive extracts (8,9).

Many other stages of manufacture involve potentially hydrolytic conditions (raised temperatures, high and low pH) and it is the art of the gelatin manufacturer to minimize degradation at these stages in order to produce a gelatin of high quality and of the required physical characteristics. Even so, because of the variable nature of the raw materials as supplied, batch differences occur in the final gelatins (9) and blending is often necessary to ensure a product of more uniform physical properties.

Hard capsule gelatins are required to be of particularly high quality and are subject to stringent **specifications** of physical properties, impurity content and microbial contamination. (See Section 1.3.1).

1.2 COLLAGEN AND GELATIN STRUCTURE

1.2.1 The Molecular Organisation of Collagen and Gelatin

The molecular composition and structure of collagen and gelatin have been extensively studied and reviewed. A short resumé is provided here but more comprehensive accounts are to be found in references 11-17.

The amino acid compositions of mammalian collagens are remarkably similar and the resultant gelatins differ only slightly in composition from their parent collagens. In comparison with other proteins, collagen possesses all the commonly-occurring amino acids (with the possible exception of tryptophan and cystine) but contains unusually large proportions of glycine (33%), alanine (11%) and

proline and hydroxyproline (22% total), and less tyrosine, methionine and histidine (<1% each).

The peptide chain in collagen has a molecular weight of about 95,000 Daltons and glycine occurs at every third residue. It is known to be regularly subdivided into regions of mainly polar and apolar amino-acid sequences, the latter being characterized by the recurring tripeptide sequence **Glycine-Proline-X**, (X can be any other amino acid residue, frequently hydroxyproline). The regular glycine spacing and tripeptide sequence have a profound effect on the preferred conformation adopted by the chain, which is a tight, left-handed helix of about three residues per turn with a pitch of 9.8. This conformation is energetically favoured because peptide bond angles and distances are close to their optimal values and amino acid side chains may be accommodated without distortion. It is further stabilized by:

A) Steric hindrance to rotation provided by the regular placings of the imino-acid residues, proline and **hydroxyproline**.

B) The aggregation of three polypeptide chains in a parallel fashion into a super helix (or triple helix) which itself has a gentle right-handed turn of pitch 86.8. The occurrence of glycine every third residue enables the polypeptide chains to be sufficiently close for inter-chain hydrogen bonding to stabilize the structure: one to two bonds occurring every three residues. Covalent inter-chain bonds infrequently occur but their number increases with age of the tissue.

When it is subjected to mild heat or reagents that break only hydrogen bonds, the triple helix collagen 'molecule' may be dissociated into a series of fractions. These comprise the intact polypeptide chains (termed the α fraction), dimers linked by a single covalent bond (β fraction), trimers (γ fraction) and various higher oligomers. Whilst two of the polypeptide chains in bone and skin collagen are very similar (α_1 type) the third (α_2 type) has been shown to differ **significantly** in amino acid composition and sequence.

There is a higher level of organisation beyond that of the triple helix structure. Electron microscopy of native collagen has shown the ordering of triple helices into linear aggregates or fibrils (18-20). These exhibit a characteristic banding pattern of period 670 Å due to the arrangement of collagen molecules within the fibril. It is considered that the polar regions of the polypeptide chain are responsible for maintenance of fibril structure whereas apolar areas are primarily involved in triple helix **stabilization**.

Gelatin is very similar to collagen in amino acid content but differs in being composed of not only α -chains and their oligomers but also the breakdown products of these molecules (the 'peptides'). This is a consequence of the hydrolysis of peptide bonds and covalent crosslinks in the manufacturing process. Below the helix-coil transition temperature (**HCTT**), which occurs between 35 and 40°C (36), gelatin in solution or in the gel state can reassemble each of the levels of collagen structure described above.

However, because of the molecular **fragmentation** in gelatin, renaturation can only be incompletely achieved.

1.2.2 The Structure of Gelatin Films

The structural entities described above have all been identified in mature "cold dried" gelatin films, that is, films dried below the HCTT. The polypeptide intramolecular helix imparts strong laevorotatory power (28), X-ray diffraction studies give patterns consistent with triple helix structure (21, 22) and electron microscopy reveals structures of the diameter of the triple helix, aggregated in a fibril-like arrangement (29). The larger fibril diameters observed in films (100 - 200 μ) compared with those of gels (60 μ) have been attributed to further aggregation on drying.

Gelatin film possesses an anomalously high negative specific rotation which is not due to greater helical content (24, 25) but to the axes of triple helices becoming coplanar with the film as it contracts during drying (26,27). Optical rotation is therefore a sensitive measure of renaturation but is complicated by an orientation factor. The relative contributions of the two may be separated (27,33).

Calorimetry (25,29), infra-red spectroscopy (23,30) X-ray diffraction (23,32) and anisotropy of swell (31) have also been used to indicate the degree of structural order in films. In contrast, films dried from solution above the HCTT ("hot dried film") exhibit an amorphous X-ray diffraction pattern, show no DSC peak for melting of structural order and have a specific rotation value no greater than that of the hot solution. It is considered

that gelatin is mostly in a random coil **configuration** and that little structural order exists in these films (24).

Hard capsules are dried below the HCTT and this work will be concerned only with "cold dried" films.

The presence of the many levels of ordered structure within the film gives gelatin a toughness and high mechanical strength among biopolymers (34). This, in combination with the solubility characteristics, cheapness and lack of toxicity and taste, makes gelatin a good material for the production of hard capsule shells. The only major disadvantages are moisture sensitivity and susceptibility to microbial growth. Patents have been granted for the manufacture of hard capsules from other polymeric materials (35), but these have not proved popular.

1.3 HARD CAPSULE MANUFACTURE

1.3.1 The **Manufacturing** Process

The first successful commercial manufacture of the two-piece hard gelatin capsule was in 1874 by F.A. **Hubel**, a pharmacist in Detroit (37). The methods involved have changed little since then, except in the introduction of automated machinery and factory air-conditioning.

Pharmaceutical gelatin supplied for hard capsule manufacture must meet exacting quality control **specifications** of physical and chemical properties. The most important of these and their influence on the resultant capsule are listed in Table 1.2. In many countries there are **pharmacopoeial** specifications for capsule gelatin (44). Three types are commonly used: acid and lime pretreated ossein gelatin and

Property	Importance
Viscosity	Controls the thickness of pin coating and therefore of the capsule shell.
Gel Strength	An indication of film strength
pH	Stability of gel strength and viscosity. Dye colour
SO ₂ content	High amounts cause dye fading
Particle size	Uniform rate of solution
Ash content	High amounts cause opacity of the finished capsule
Colour and clarity	Uniformity of shade
Bacterial count	Viscosity loss. Bubble liberation. Putrefaction.

TABLE 1.2 The Properties of Gelatin and their
Importance in Hard Capsule Manufacture (2)

acid pigskin gelatin. They are blended in proportions according to their availability, market price and the previous experience of the **manufacturer**. Eli Lilley of Basingstoke use a blend of 5:1 limed to acid processed gelatins (39). Recently a high quality, low bloom gelatin has been supplied as a low cost "filler" for incorporation up to 10% in the blend (40).

Manufacturing plants have been described in detail in references 2, 38, 41 and 42. For successful manufacture, close environmental control is necessary and the whole factory is temperature and humidity controlled. Eli Lilley maintain conditions at 42% R_H and 23°C (2) and the following account is based on their production method. Other companies use closely similar **manufacturing** processes but operating conditions may differ (42). The production cycle consists of preparing the gelatin solution, casting onto metal mould pins, drying, stripping, trimming, assembly, printing, inspection and packing.

Gelatin grist is swollen in demineralized **water**, melted and stirred for 3 hours at 60°C and allowed to degas at 60°C for a further 5-7 hours. The concentration of the solution is approximately 30% w/w. Dyes may be added up to 3%, titanium dioxide up to 2% (**for** opaque capsules) and sodium dodecyl sulphate (wetting agent) up to 100 ppm. The solution viscosity, which controls the capsule wall thickness, is measured and adjusted and the solution vats wheeled out to the capsule making machine.

Rows of cooled lubricated steel moulding pins are dipped into the hot gelatin solution, removed and rotated to spread the solution evenly around the pin. The coated pins are fed into a succession of drying chambers increasing in temperature from 23 to 28°C. Drying takes 45 minutes and the capsules are stripped from the pins, trimmed to size and the two halves assembled and printed. The capsules contain approximately 3% excess moisture; further drying occurs on storage.

The new capsules are visually examined against a bright background for defects. The types of defects that may occur are listed in Table 1.3. They are packed in lined drums to prevent moisture changes on storage. The optimum humidity range for storage is 30 - 40% R_{rH} (45-47), above which capsules swell and lose rigidity and below which shrinkage and brittleness ensues.

1.3.2 The Problems of Hard Capsule Manufacture Related to Gelatin

The ideal gelatin for hard capsule production is inexpensive, and on blending produces capsules that are fault-free, colourless, clear, strong, flexible and dry to the optimum degree under the standard operating conditions. Unfortunately, gelatins differ and their performance on capsule-making machines and the quality of the finished capsule cannot be predicted by the routine quality control tests on the raw materials. In practice 'good' and 'poor' gelatins are encountered of which the latter cause problems in the finished capsules evident as:

Major Defects	Minor Defects
Cracked	Bubbles
Cuttings inside	Corrugations
Double dip	Flat spots
Holes	Grease
Mashed	Heavy ends
Short bodies	Pin marks
Splits ?	Scrapes
Telescoped	Short caps
Thin ends	Specks
Trimmings 7	Splits
Uncut bodies	Starred
Bad edges	
Crimps	
Dented Ends	
Double caps	
Long bodies	
Long caps	
Long joined	
Oily	
Thin spots	
Uncut caps	
Unjoined	

TABLE 1.3 Visual defects in Hard Gelatin Capsules (43)

Major defects: cause failure as a container for
 medicament or problems with filling.

Minor defects: have no adverse effect on performance
 but are blemishes that make the capsule
 visually imperfect.

- a) an increased incidence of splitting and cracking-type defects thought possibly a result of overdrying during the standard 45 minute drying cycle.
- b) a lack of flexibility ('brittleness') which may lead to difficulties in trimming or on subsequent handling and filling.

Problems (a) and (b) can be related. An over-dried capsule will be brittle and brittle capsules may fracture when trimmed thereby increasing the number of visual defects. However there is evidence (39, 48) that different gelatins and blends may be inherently different as films, although the literature is sparse and sometimes conflicting. Significant differences are encountered in practice but supporting numerical evidence is scarce (48).

Van Hoesteler (41) states that limed ossein gelatins form 'firm but hazy and brittle' capsules in which flexibility and clarity is improved by the addition of acid derived gelatins. Other sources (40,49,50) say pigskin gelatin is the more brittle and, although its addition to limed ossein improves film qualities, there is an upper limit (50%) above which brittleness becomes an unworkable problem. The increased brittleness of acid processed gelatins is attributed to different film drying characteristics (50). The inclusion of 30% or more acid ossein gelatin is claimed to improve flexibility (49). One capsule maker (39) finds a 5:1 limed:acid gelatin ratio an ideal blend but this may not be true of others using different operating conditions.

There is a dearth of information relating the physico-chemical properties of different gelatins to their performance during hard capsule manufacture, and consequently there is at present, no testing regimen suitable for detecting 'poor' gelatin batches before they are put into production. In addition, the influence of gelatin type on the physical properties of gelatin films has received little attention.

1.4 THE AIMS OF THIS WORK

It is proposed to examine some physical properties of gelatin films pertinent to the manufacture and quality of hard gelatin capsules and the problems thereof. This will include studies of film drying rates, equilibrium moisture contents, mechanical properties and frictional properties of a number of well-characterized gelatin batches, in relation to gelatin type and known performance on the capsule manufacturing machines.

Characterization will involve molecular mass distribution analysis, isoionic point determination and tests for impurities not detected by routine quality control tests. The molecular mass distribution is an important modulator of gelatin physical properties and has been related to, for example, setting time, gel rigidity and viscosity (51).

Film drying rates are claimed to differ with gelatin type and might also provide an insight into the behaviour of 'poor' gelatin batches on the capsule-making machine and film brittleness. Other properties, such as mechanical strength, being equal then the use of a greater proportion of a faster-

drying gelatin in the blend would confer advantages of time-saving in the manufacturing process and more use of the cheaper acid processed gelatins would reduce raw material costs.

Equilibrium moisture content (EMC) values indicate the water binding capacity of a film. Inherent differences in EMC for different gelatins under the same conditions, may result in different mechanical behaviour, as water is a powerful plasticizer of gelatin films (52). Capsules that contain low levels of moisture are known to be excessively brittle (47).

The investigation of mechanical properties by tensile testing will determine what (if any) differences occur between types and blends of gelatin as mature films and may reflect structural differences within the film.

Finally, the introduction of a 'glide test' for the determination of coefficient of friction, as a specification for raw materials for hard capsule manufacture indicates that this property is also one recognised as giving rise to problems (40). Varietal differences in film frictional coefficients will be investigated.

CHAPTER II

GENERAL EXPERIMENTAL

2.1 THE GELATIN USED IN THIS WORK

The batches of hard capsule grade gelatins used in this work were the products of five different **manufacturers**. They are numbered 1 to 10 and details are given in Table 2.1

Batch 1 was a limed ossein gelatin used in previous studies (53). Batches 1,2,3 were limed osseins known to produce satisfactory capsules whereas 4,5,6 were 'poor' batches that consistently produced **unsatisfactory** capsules.

Batch 10 was a **60-Bloom** alkali ox-hide gelatin supplied as a low cost 'filler' for inclusion up to 10% in the blend.

2.2 THE PREPARATION OF THIN GELATIN FILMS

2.2.1 Possible Methods

Thin gelatin films, of thickness 0.05 - 0.5 mm have been prepared from aqueous solutions by the following methods:

- (a) Casting dilute solutions in shallow moulds and subsequently drying (21,26,54)
- (b) Pouring a hot concentrated solution down an inclined surface (34)
- (c) Spreading a hot concentrated solution using a hand-held chromatography spreader (56).

Batch	Type	Manufacturer
1	limed ossein (good capsules)	1
2	limed ossein (good capsules)	2
3	limed ossein (good capsules)	2
4	limed ossein (poor capsules)	3
5	limed ossein (poor capsules)	3
6	limed ossein (poor capsules)	4
7	acid ossein	1
8	acid ossein	1
9	acid pigskin	5
10	alkali ox-hide (low bloom)	1

Table 2.1 The Batches of Hard Capsule Grade Gelatins
used in this work

The substrate was a plastic, metal or siliconed glass surface from which the film was then stripped off. For very thin films a mercury surface can be used (57).

2.2.2 Apparatus

In this work gelatin films were prepared using an automated chromatography spreader (58). (Plate 2.2). This comprised two brass hoppers which were pushed along a siliconed glass plate by means of a motorized trolley at a constant speed of 10 cm.s^{-1} . The film width was 5 cm and the thickness could be altered by adjustment of the gap at the rear of each hopper.

2.2.3 Method

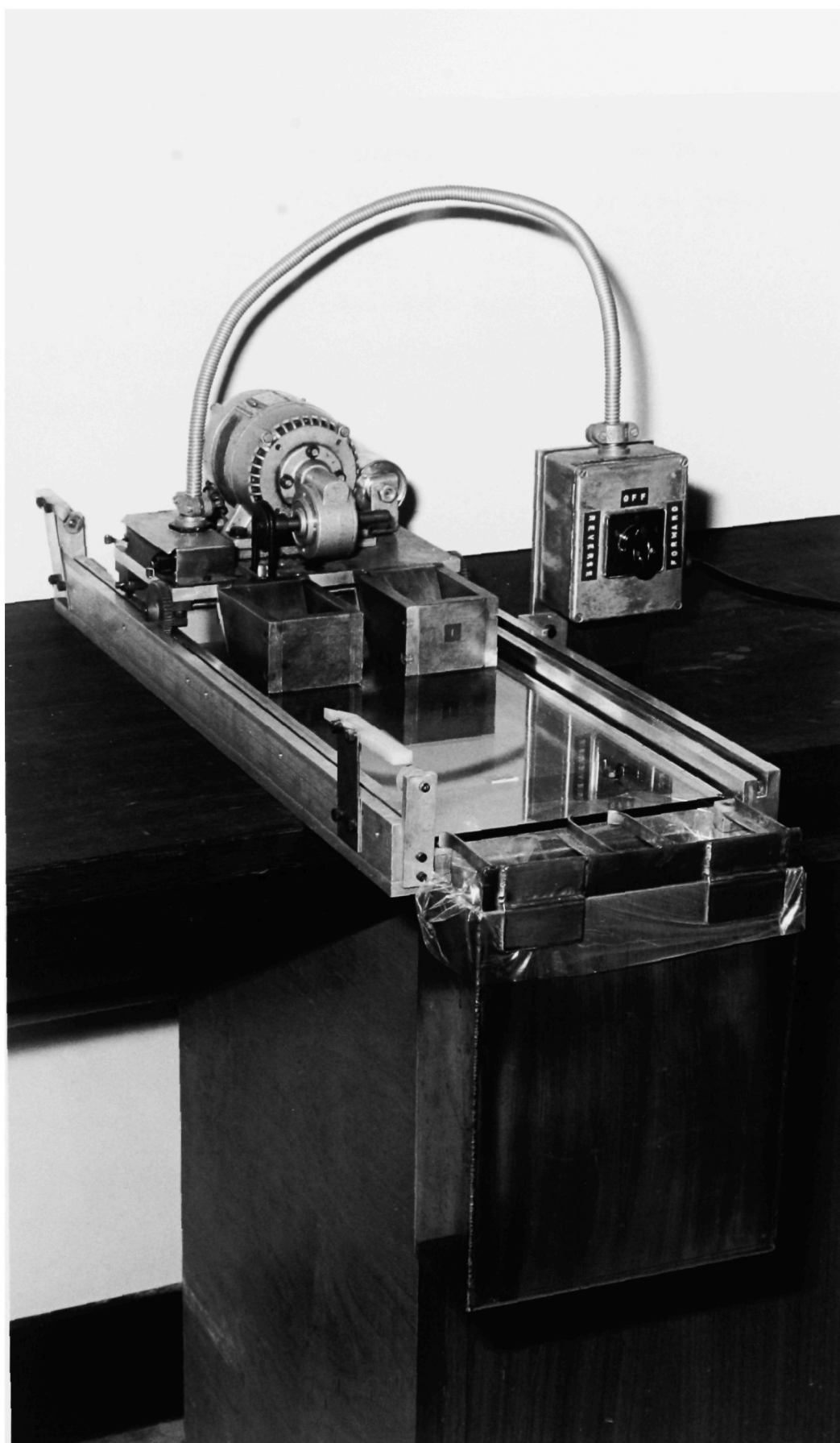
A concentrated gelatin solution for spreading was prepared in the following manner,

- A. A 25% W/W mixture of gelatin and distilled water was allowed to soak for at least three hours at room temperature in a closed vessel.
- B. The swollen gelatin was melted in a water bath at 50°C for 2 hours.
- C. The molten gelatin was mixed for 15 min. using a magnetic stirrer, and then allowed to stand 16 hours at 50°C to degas.
- D. Immediately prior to use, the solution was mixed for a further 10 min. to ensure homogeneity.

For a comparison of the degradation of the gelatin solutions prepared in this way with that undergone by gelatin solutions prepared in industry for the manufacture of hard capsules see section 3.3.

PLATE 2.2

Automated Chromatography Spreader for Preparing Thin
Gelatin Films



Films were spread at room temperature ($20^{\circ} \pm 1^{\circ}\text{C}$) and when dry films were required, were dried under cover at $20 \pm 1^{\circ}\text{C}$ and $40 \pm 3\% \text{ R}_\text{H}$ for 24 hours in the humidity and temperature controlled room.

Casting conditions were kept constant as the nucleation and growth of triple helical areas is affected by temperature (60).

Examination for optical birefringence (59) on clear, flat pieces of film failed to produce evidence of molecular orientation arising from the spreading procedure. For uniaxial mechanical testing it was important that films were isotropic.

2.3 THE DETERMINATION OF FILM MOISTURE CONTENT

2.3.1 Method

The method was that of the British Standard Method (61) with one adaptation. The weighed film was placed inside a folded sheet of aluminium foil (a) and dried at $105 \pm 0.5^{\circ}\text{C}$ for 18 hours in an oven (b). On removal from the oven, the foil was pressed against the sample, folded to seal, and the foil and **sample** and foil alone reweighed. The percentage w/w moisture content was calculated on a wet weight basis (69) thus:

$$Y = 100 \frac{(M - M_D)}{M}$$

Y : % moisture content

M : original sample weight

M_D : sample weight after drying

(a) Aluminium Foil, Safeway Food Stores **Ltd., London.** U.K.
 (b) Thermostat Oven, Tomson & Mercer Ltd., London. U.K.

2.3.2 The Systematic error involved in the Moisture Determination Method.

The method has been used to elucidate small differences in the moisture contents of different gelatins (Chapter 5) therefore an attempt was made to estimate the degree of systematic error involved in this procedure.

Figure 2.3 shows the moisture regain of a typical-sized piece of gelatin film upon removal from the oven , both wrapped in foil and unwrapped. The foil itself did not regain moisture.

The wrapping and weighing of eight samples (the maximum subjected to moisture determination at any one time) was accomplished in three minutes. Extrapolating the curves to zero time, the unwrapped gelatin would gain 3.7mg (0.46%) and wrapped 2.0mg (0.35%) in three minutes. The true systematic error from this source probably lies in an extrapolation between these two, using the 'wrapped' curve after the initial 20 seconds and is likely to be of the order of $\sim 0.5\%$ w/w.

The very rapid regain of moisture immediately after removal from the oven could lead to differences between the first and eighth sample to be wrapped. For high precision experiments on repeated samples (Chapter 5) this effect was randomised by wrapping samples in rotation.



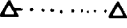
2.3.3 Method Reproducibility and Accuracy

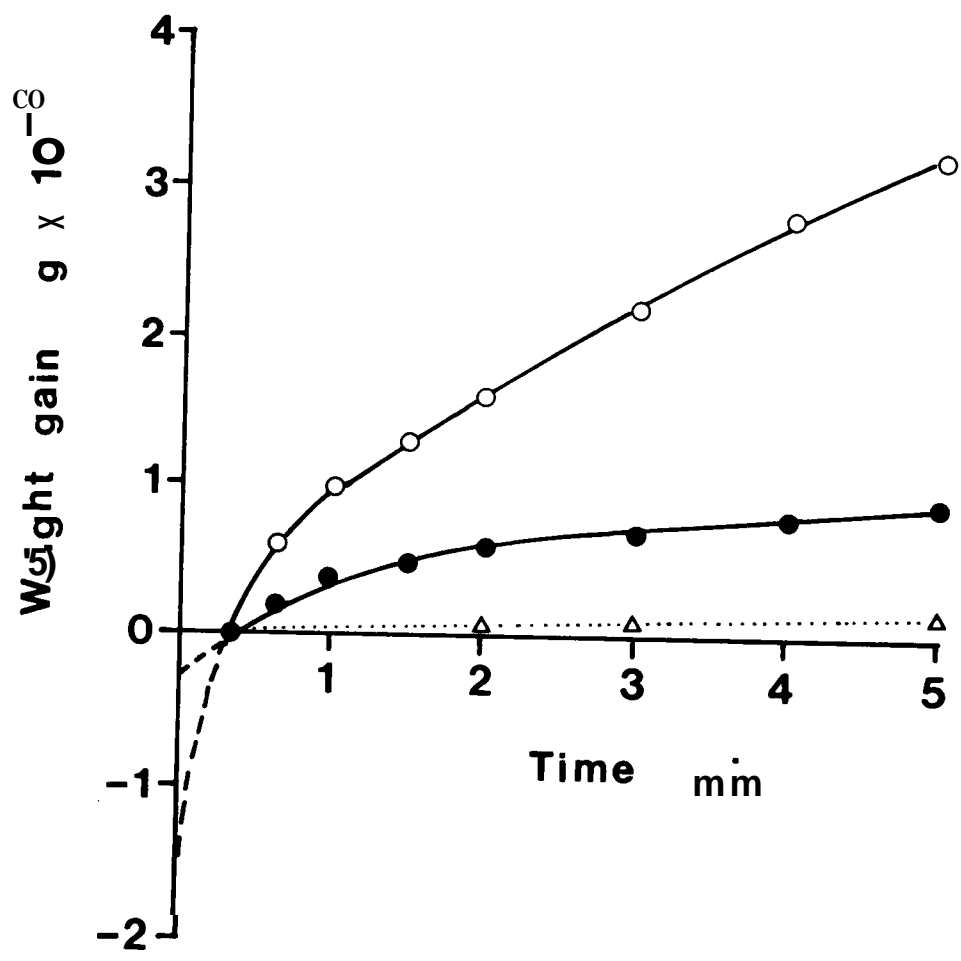
Eastoe and Williams (62) have found that for gelatin films the reproducibility of the British Standard Method

FIGURE 2.3

The Moisture Regain of Dehydrated Gelatin Film on
exposure to an Atmosphere of 40% R_H at 20°C

Sample size approximately 125 x 0.2 mm
Weight 0.58 g. Samples were removed from oven at
t = 0 and wrapped at t = 0.3 min.

	Unwrapped film
	Wrapped film
	Aluminium foil only (area 230 x 100 mm)



was good ($\pm 0.1\%$. $n=4$).

The results varied only slightly with oven temperature but more so with the humidity inside the oven. An attempt was made to control this variable by keeping the oven in a humidity controlled room at $40 \pm 3\% R_H$.

The method gives results that are only 0.1 to 0.3% higher than drying to constant weight (three days at 105°C (63) although the latter method may itself be unreliable due to the decomposition that occurs on prolonged heating (15).

2.4 HUMIDITY CONTROL

2.4.1 Saturated salt solutions

Inorganic salts, used as saturated solutions, and their resulting humidities are detailed in Table 2.4. As used for conditioning film strips, the salts were maintained as moist pastes or slurries by the regular removal of supernatant liquid or the addition of fresh saturated solution (64, 65).

There is disagreement in the literature over the precise humidity provided by saturated lithium chloride solution at 20°C . BS3718 (64) gives a value of $12\% R_H$ based on measurements made by the National Physical Laboratory (66) whereas other sources (67,68) cite values of 15%.

Measurements in this work made relative to other salt solutions, using the instrument described in section 2.4.3 gave readings close to $12\% R_H$ and this value has been used in this work.

SATURATED SALT SOLUTION	GRADE	TEMPERATURE			
		20°C	25°C	30°C	35°C
		RELATIVE HUMIDITY %			
LITHIUM CHLORIDE	Reagent	12	12	12	12
MAGNESIUM CHLORIDE	Reagent	33	33	33	33
POTASSIUM CARBONATE	Reagent	44	43	43	43
SODIUM DICHROMATE	Reagent	55	54	53	52
AMMONIUM NITRATE	Analar	65	62	59	55
SODIUM CHLORIDE	Analar	76	75	75	75
POTASSIUM CHLORIDE	Analar	86	85	85	84

TABLE 2.4 Inorganic salts used for Humidity Control (64)

BDH Chemicals Ltd., Poole, Dorset, U.K.

Desiccators, for the individual conditioning of film strips were built. Their design is shown in Figure 2.5.

2.4.2 The Humidity and Temperature-Controlled room (HTCR)

The HTCR was equipped with a Qualitair Model 230TC (a) unit which circulated, filtered and conditioned room air. With the provision of additional heat and humidity sources for use at the highest or lowest humidities, the air was maintained at $20 (\pm 1)^{\circ}\text{C}$ and the unit cycled the relative humidity about the required value $\pm 3\%$.

Spot measurements of temperature and relative humidity were taken using an Aspirating Wet and Dry Bulb Hygrometer (b) and the room was continually monitored by means of a thermohygrograph (c).

2.4.3 Humidity Monitoring Instrument for Drying Apparatus

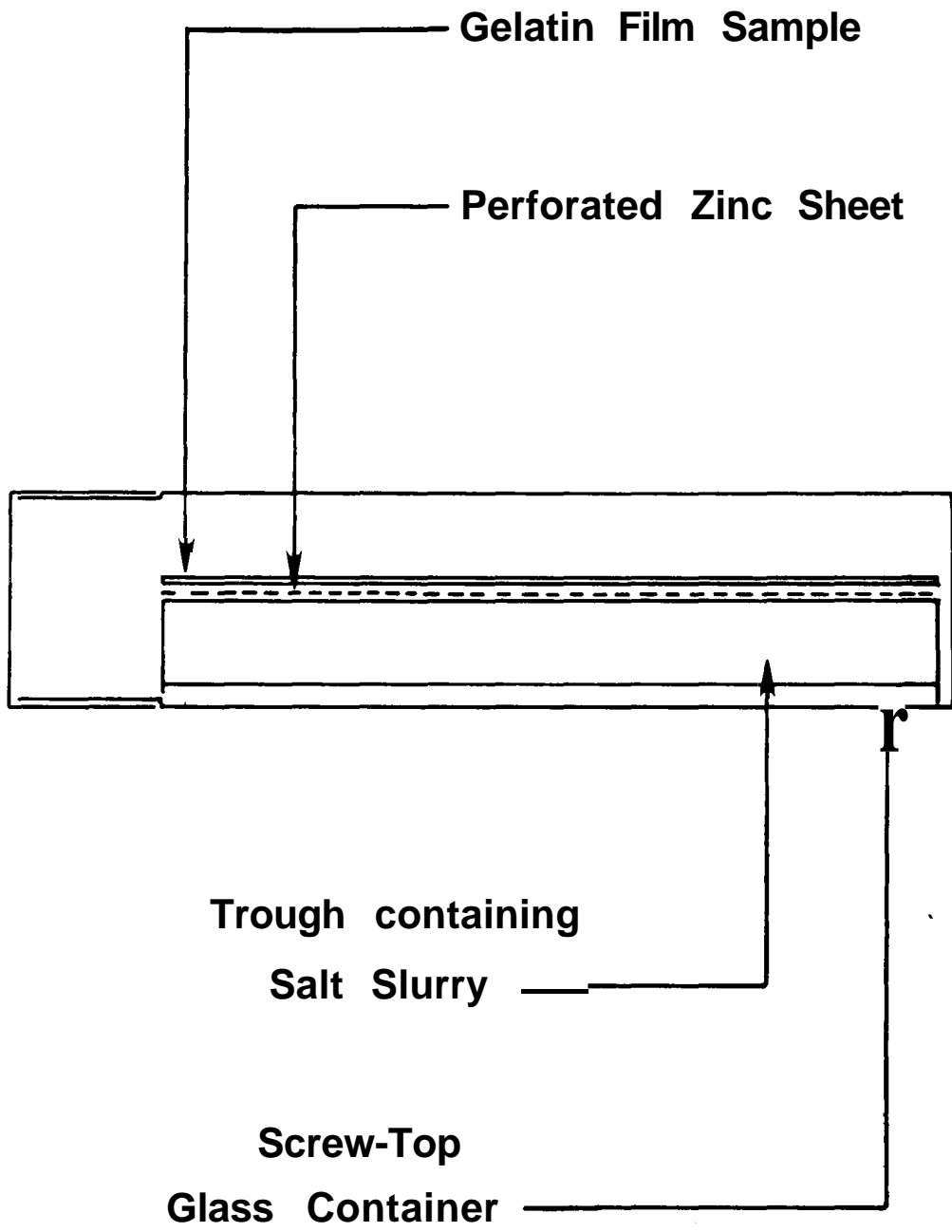
Air inside the drying apparatus (section 4.2) could not be monitored by means of wet and dry bulbs as a wet bulb was found to release too much water vapour into the system.

An instrument was therefore used which employed a sensor, the capacitance of which varied according to the water content of the dielectric. This instrument was home built and the circuit diagram is shown in Figure 2.6. R_H values could be read from the meter to better than 1%.

-
- (a) Qualitair (Air Conditioning)Ltd. Castle Road, Eurolink, Sittingbourne, Kent, ME10 3RH.
 - (b) Model 2026 } Casella Ltd., Regent House, Britannia
 - (c) Model T9154 } Walk, London N1.

FIGURE 2.5

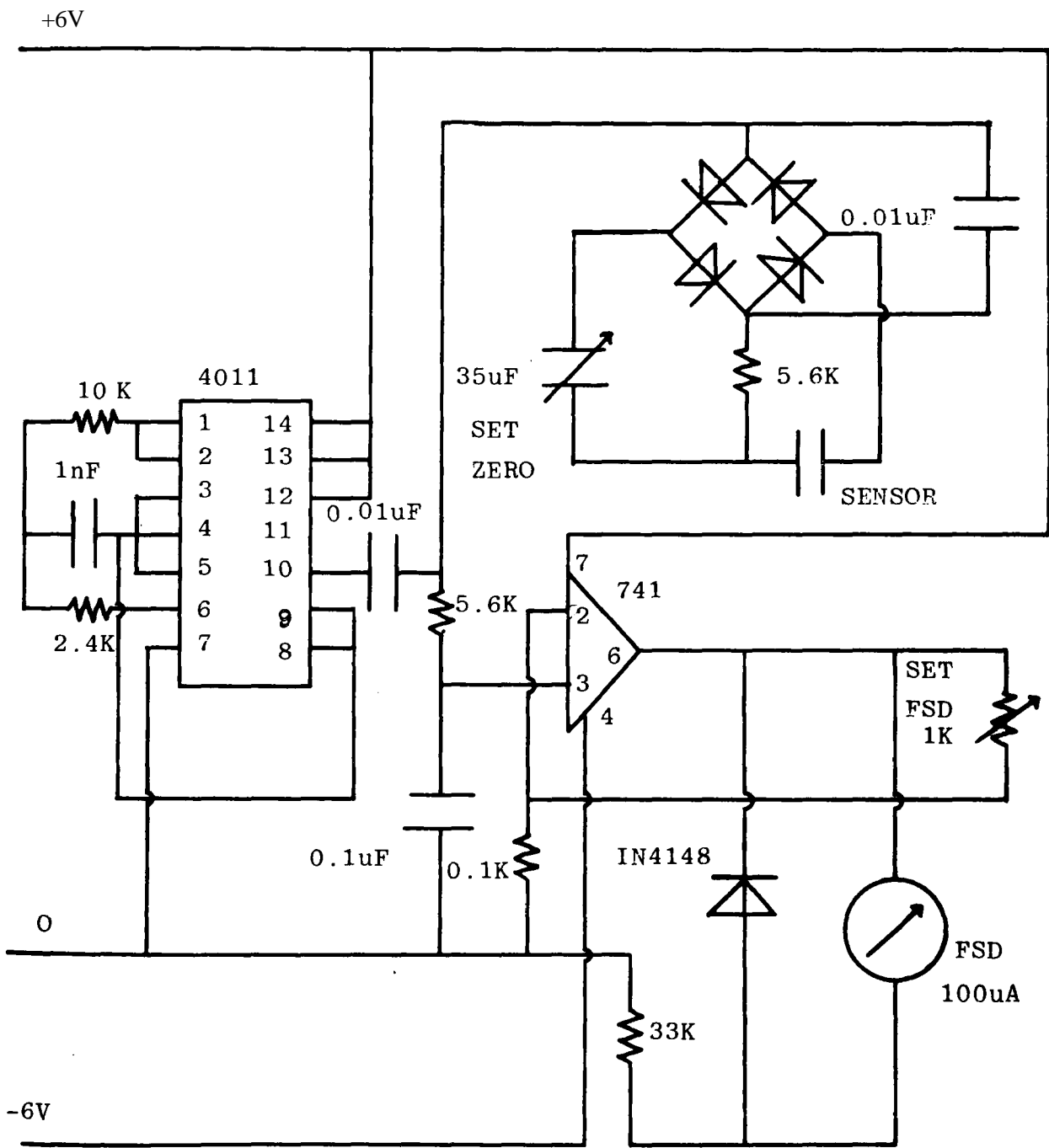
The Design of Individual Desiccators for Conditioning
Gelatin Films



The instrument was calibrated and checked against a range of saturated salt solutions before use and was accurate and responded rapidly within the range 20 to 80% R_H . At lower relative humidities the instrument took several hours to reach a steady reading. Above 80% R_H the instrument was unreliable.

FIGURE 2.6

Circuit Diagram of the Humidity Measuring Instrument used
in the Drying Apparatus



CHAPTER III

CHARACTERIZATION

3.1 MOLECULAR MASS DISTRIBUTION BY POLYACRYLAMIDE

GEL ELECTROPHORESIS

3.1.1 Introduction

Gelatins are highly polydisperse. A commercial ossein gelatin has been reported to contain species of

3 7

molecular mass 4×10^3 to 1×10^7 Daltons (70). Therefore methods such as osmometry, light scattering and viscometry that define average molecular weights (71) are of limited value, and do not indicate the discontinuous nature of the molecular mass distribution (MMD). Preparative fractionation by coacervation has been used (72) but the fractions themselves are polydisperse (79,86).

Techniques available to separate heterogeneous protein systems include **ultracentrifugation**, gel permeation chromatography and gel electrophoresis (73). The first two methods have successfully separated α , β and γ components of denatured collagens (74) but, presumably because of the more continuous nature of the MMD, show poorer resolution of gelatin components than gel electrophoresis (8,51,75).

The inert nature, lack of thermolability and ability to be prepared in a variety of well-defined pore sizes has

led to the widespread use of polyacrylamide gels as electrophoretic substrates (76,77). Separation is achieved by a combination of electrophoretic effects and "molecular sieving" by the pores of the gel. Trishna has also reported good separations of gelatin using a cellulose substrate (82). Fujii and Katamura (80) using a tris buffer system at pH 7.5 separated two limed gelatins sufficiently well to identify α , β and γ components. Bartley and Marrs (78,79) obtained better resolution using a formate buffer at pH 3.7 and established that the separation of gelatin components by polyacrylamide gel electrophoresis (PAGE) was primarily on a molecular mass basis, rather than one of charge. **However, it** is the work of Tomka and co-workers (51,75,81) that has established PAGE as a method suitable for the quantitative estimation of gelatin components.

3,1.2 Apparatus and Reagents

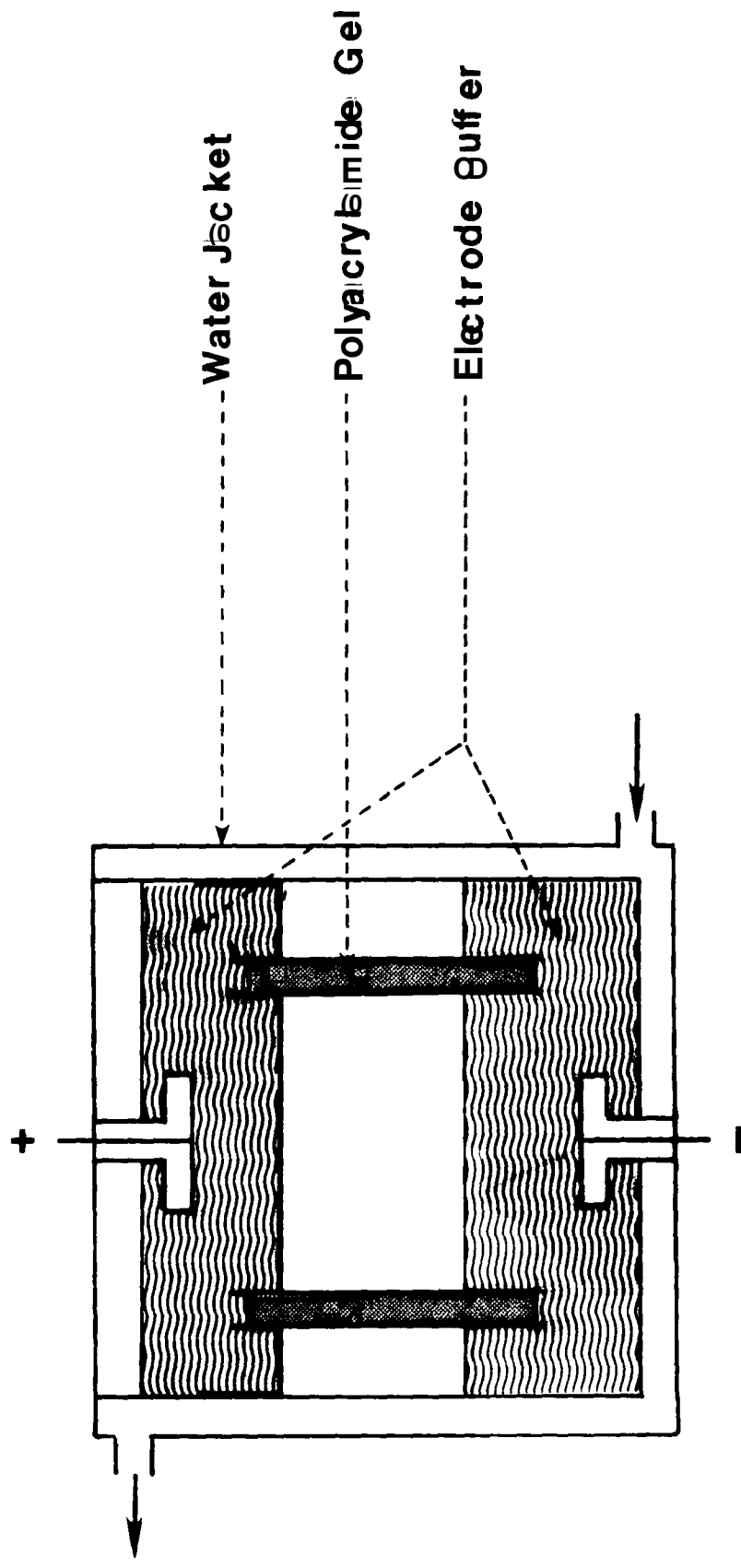
ELECTROPHORESIS CELL

Disc Electrophoresis Apparatus
Cell maintained at **40°C**. "Vokam" stabilized power supply.

Shandon Southern Products Ltd.,
Runcorn,
Cheshire. WA7 1PR
U.K.

: FIGURE 3.1

A Diagram of the Electrophoretic Cell



DESTAINER

Transverse Gel Rod
Destainer Mk.III. **SAE-2751**

Shandon-Southern Products Ltd.,
Runcorn,
Cheshire. WA7 1PR
U.K.

DENSITOMETER

Chromoscan fitted with 20D lamp, 0503 aperture,
filter 5-042 optimum for Napthalene Black.

Joyce **Loebel**,
Gateshead,
Tyneside.
NE11 OQW
U.K.

REAGENTS

The following obtained from:

BDH Chemicals Ltd.
Poole,
Dorset.
BH12 4NN
U.K.

Glacial Acetic Acid)	
Glycine		
Sucrose		
Potassium Hydroxide Solution M		Analar grade
Ammonium persulphate		
NNN'N' Tetramethyl ethylene- diamine (TEMED))	
Napthalene Black 12B)	Electrophoresis grade

REAGENTS

The following obtained from:

Bio-rad Laboratories,
Watford,
Herts.
U.K.

Acrylamide	}	Electrophoresis grade
Bisacrylamide		

The following obtained from:

Dr. I. Tomka
Ciba-Geigy
Fribourg.

Nonyl phenol

WATER Deionized. Conductivity <2 milli mhos.cm³

ELECTRODE BUFFER

0.015M glycine adjusted to pH 3.75 with acetic acid.

3.1.3 Method

The general method of gel-forming and electrophoresis followed was that of Tomka (51,83) with some **modifications**.

Polyacrylamide gels were formed at 20°C in glass tubes under nitrogen by the method of Tomka. The pore-size is controlled by the concentration of initiator (ammonium persulphate), activator (TEMED) and acrylamides present (84). Two hours after casting, the tubes were mounted in the electrophoresis cell. 10 µl of 0.5% w/w gelatin in 20% w/w sucrose solution was applied to the top surface of the gel

and the electrophoresis performed at 10 mA (80-100v) for 30 minutes followed by 20 mA for 10 minutes. 5ml of 0.7% naphthalene black in electrode buffer was added to the lower chamber and the electrophoresis continued at 20 mA for 20 minutes, then 12 mA for approximately 70 minutes until the dye had emerged from the top of the gel rods. The rods were removed from the glass tubes and transversely destained in 7% ^w/w acetic acid solution at 800 mA for 13 minutes. Destaining in this apparatus was non-uniform and the operation was checked complete by means of a blank gel. Rods were mounted in the densitometer taking care to remove air bubbles which give rise to false peaks. Optimal instrument settings were found to be gain 4, chart sample ratio 3:1, normal mode, with wedge C or D.

After identification, densitogram peaks were integrated using the blank gel as a baseline. This usually coincided with the lowest point of each densitogram.

Each gelatin was run on 2.5 and 5% gels in order to resolve the higher and lower end of the molecular mass spectrum. The most important factor in the quality of the separations was found to be the formation of the upper gel meniscus by the monomer: water interface during casting. Any degree of mixing resulted in poor resolution. Separations were performed in triplicate and the best one selected for the densitogram. Those showing badly distorted, trailed or diffuse bands were discarded.

3.1.4 Results

An **I.A.G.** limed ossein gelatin (DFG/Stoess 2766) was run as a typical limed ossein, and as a check on experimental accuracy. Densitograms are shown in figures 3.2 and 3.3. The peaks were assigned to their respective components using published mobilities relative to that of the **α -peak** (Table 3.4). Species of molecular mass below that of the a chain ($9.1 - 9.5 \times 10^4$ D) are designated 'lower peptides', those between β and γ peaks, g peptides, and between g and γ , Y peptides. Components 1 to 6 are higher oligomers and fragments of similar size whilst μ is the microgel fraction which has been reported to have a molecular mass of 2×10^6 to 2×10^7 D (81).

The 5% gel provides a better estimation of the content of lower peptides and the 2.5% gel resolves more clearly the higher components. Using the lower peptides content calculated from the 5% gel, the fractional areas were normalized with respect to α peak area and the results expressed as a percentage total area. These **values, in** comparison with those of Tomka for the same gelatin (85), are shown in Tables 3.5 and 3.6. α^1 , and α_2 oligomers and fragments were poorly resolved and they are not shown independently. When the densitogram is subdivided into many components, the agreement with Tomka is less satisfactory than using fewer fractions based on the clearest features of

the trace i.e. the fronts of the β , α and A bands. The subjective assessment of the beginning and end of each peak considerably influenced the result for the small fractions if a finer subdivision was applied. In addition, peak overlap may cause overestimation of narrow fractions. These constraints are illustrated by the large values for the narrow A component compared with those of Tomka. The reproducibility of three separate determinations on this gelatin is shown in Table 3.7.

Densitograms for an acid ossein (Croda 472) are shown in Figures 3.8 and 3.9. The absence of a prominent α peak required the parallel electrophoresis of a limed : acid ossein mixture to elucidate its position (Figure 3.10). It was not possible to use the 5% gel trace to quantify the lower peptide content because of the lack of a well defined α peak, and the high initial peak which made estimation of the total area difficult.

In limed osseins the content of lower peptides is consistently underestimated by the 2.5% gel traces and there appears no reason why this should not also apply to acid osseins. However, the results are comparable with those obtained by Tomka (Table 3.11).

The densitograms for the two other types of gelatin, the acid pigskin and the alkali hide (60 Bloom), are shown in Figures 3.12 to 3.15. Lower peptide contents were also estimated for these gelatins from the 2.5% traces alone.

FIGURE 3.2

Densitogram of the Electrophoretic Separation of a Limed
Ossein Gelatin on 2.5% Polyacrylamide Gel

Gelatin : DFG/Stoess 2766

➡ : Direction of Electrophoretic migration

The designation of fractions is explained in the text.

Optical
Density
[Arbitrary
Units]

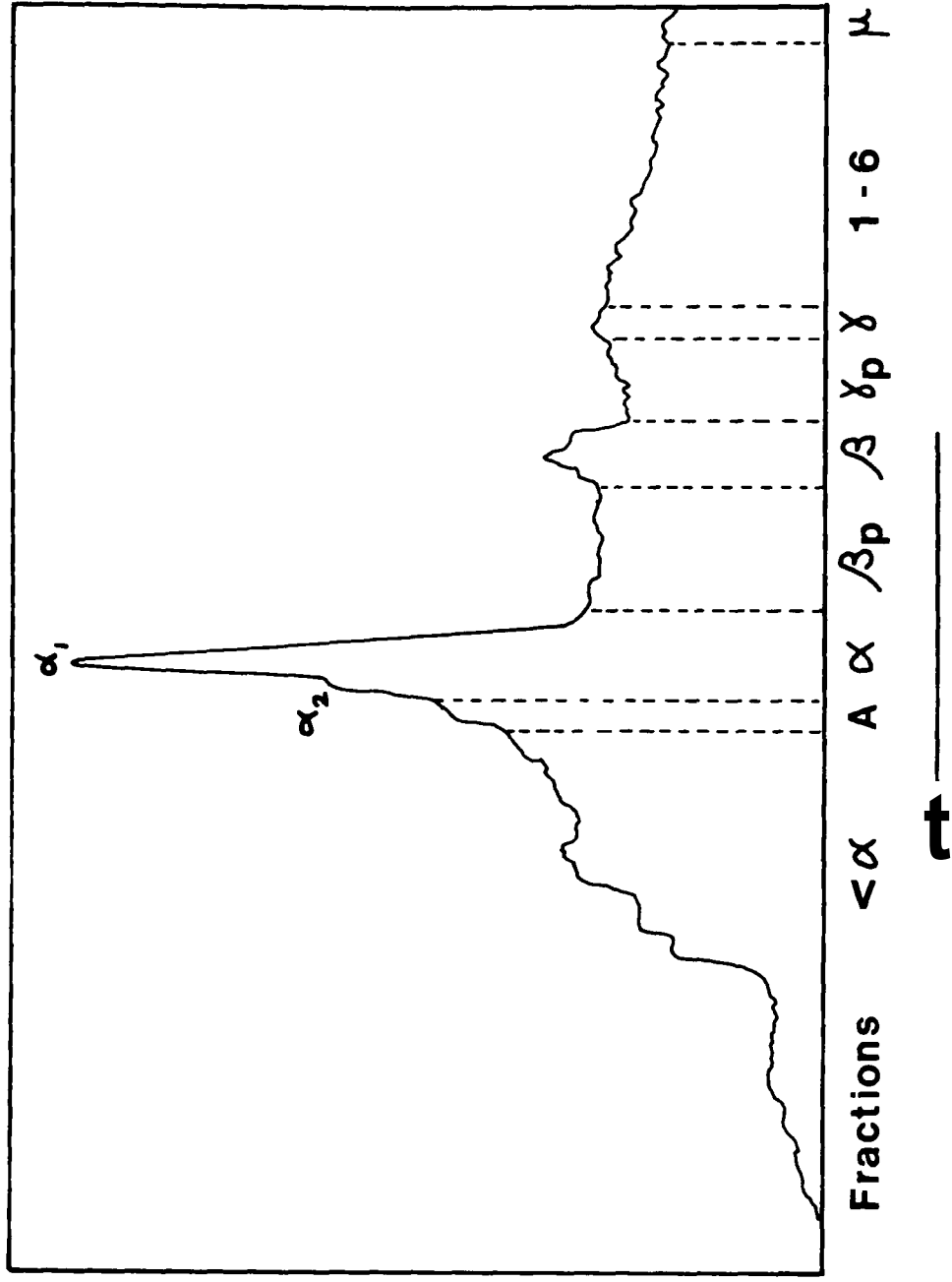
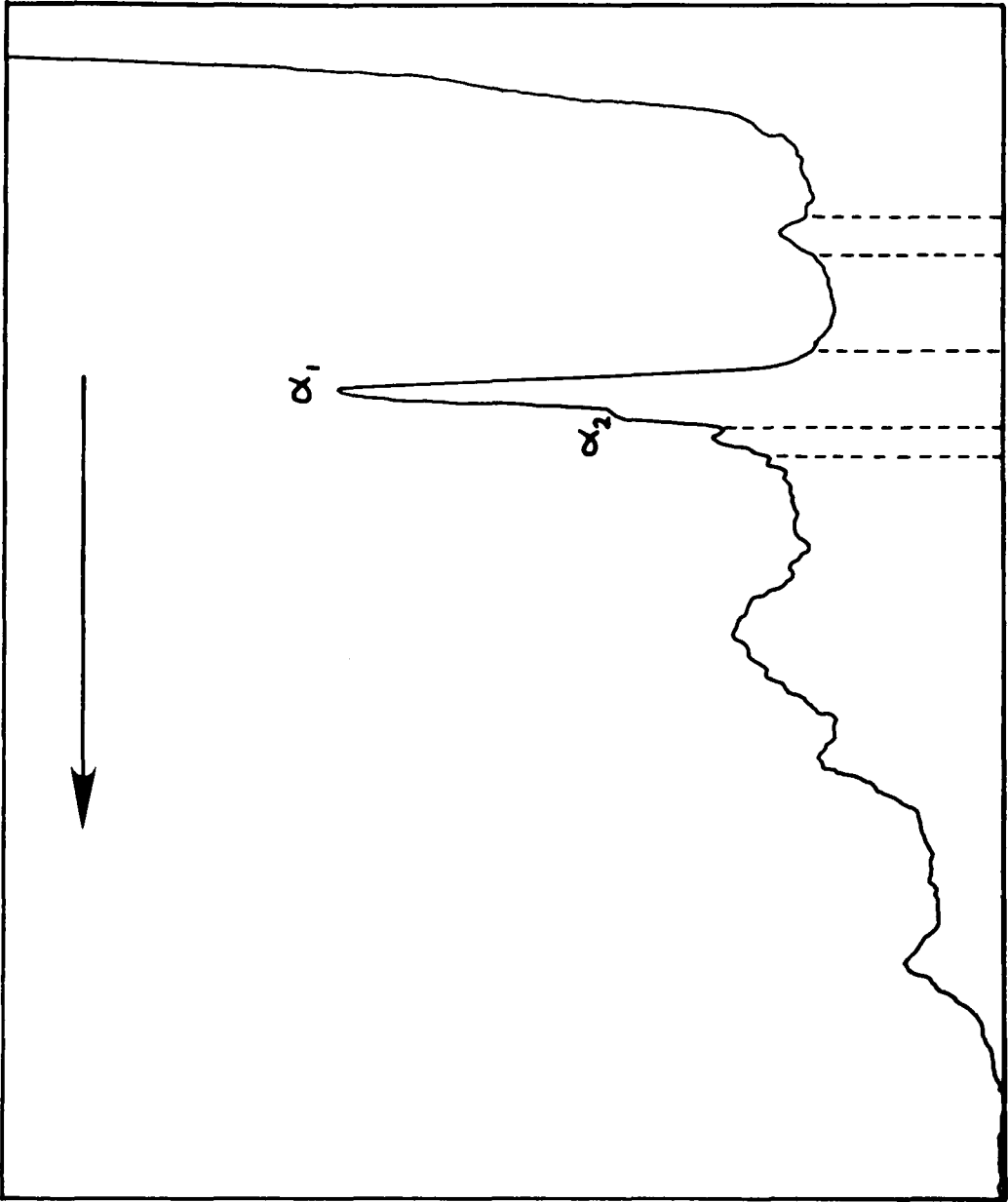


FIGURE 3.3

Densitogram of the Electrophoretic Separation of Limed
Ossein Gelatin on 5% Polyacrylamide Gel

Gelatin : DFG/Stoess 2766

➡ : Direction of Electrophoretic migration



OD

COMPONENT	D	C	B	A	α_1	β_{11}	γ	1	2	3	4	5	6	μ
$\frac{\omega_F}{\omega_{F(\alpha)}}$	1.58	1.50	1.29	1.10	1	0.87	0.47	0.88	0.280	2.50	± .7	0 ± 5	0.18	0.96
$\frac{M}{M(\alpha)}$	0.51	0.87	0.78	0.91	1	2	8	4	5	8	7	8	8	10

TABLE 8.4 Molecular Characterization of Gamma in Coagulation Parameters PAGE (52)

$\frac{\omega_F}{\omega_{F(\alpha)}}$
:
Electrostatic mobility of zig-zag component relative to ω_{α_1} of α_1 -chain

$\frac{M}{M(\alpha)}$
:
Molecular Mass of a component relative to that of ω_1 chain (9.5×10^4 daltons)

TABLE 8.5 Many sub-divisions

COMPONENT	μ	$1+1-6$	γ'	γ_{av}	β	β_{av}	s'	A	Δ
This Work	18.9		8.8	8.2	7.0	9.8	22.6	3.7	28.0
Tomka	17.3		8.7	4.4	8.9	8.9	24.2	1.7	27.8

TABLE 8.8 Fewer sub-divisions

COMPONENT	$\mu - \gamma_{av}$	$\beta + s_{av}$	a	A	Δ
This Work	28.9	16.8	22.6	8.7	28.0
Tomka	28.4	17.8	24.8	1.7	27.8

TABLES 8.5 and 8.8

The Composition of Lined Ossein Gelatin DFG/Stoess 2766 calculated from Fractional Areas of PAGE Densitograms compared with the results obtained by Tomka (85)

COMPONENTS	>g	$\beta + \beta_p$	a	A	<A
Composition (%)					
RUN 1	28,9	16.8	22.6	3.7	28.0
RUN 2	28,5	16.0	21.6	3.9	30.0
RUN 3	30,0	19.2	21.1	2.8	26.9
Range of Variation (%)	1,5	3.4	1.5	1.1	3.1

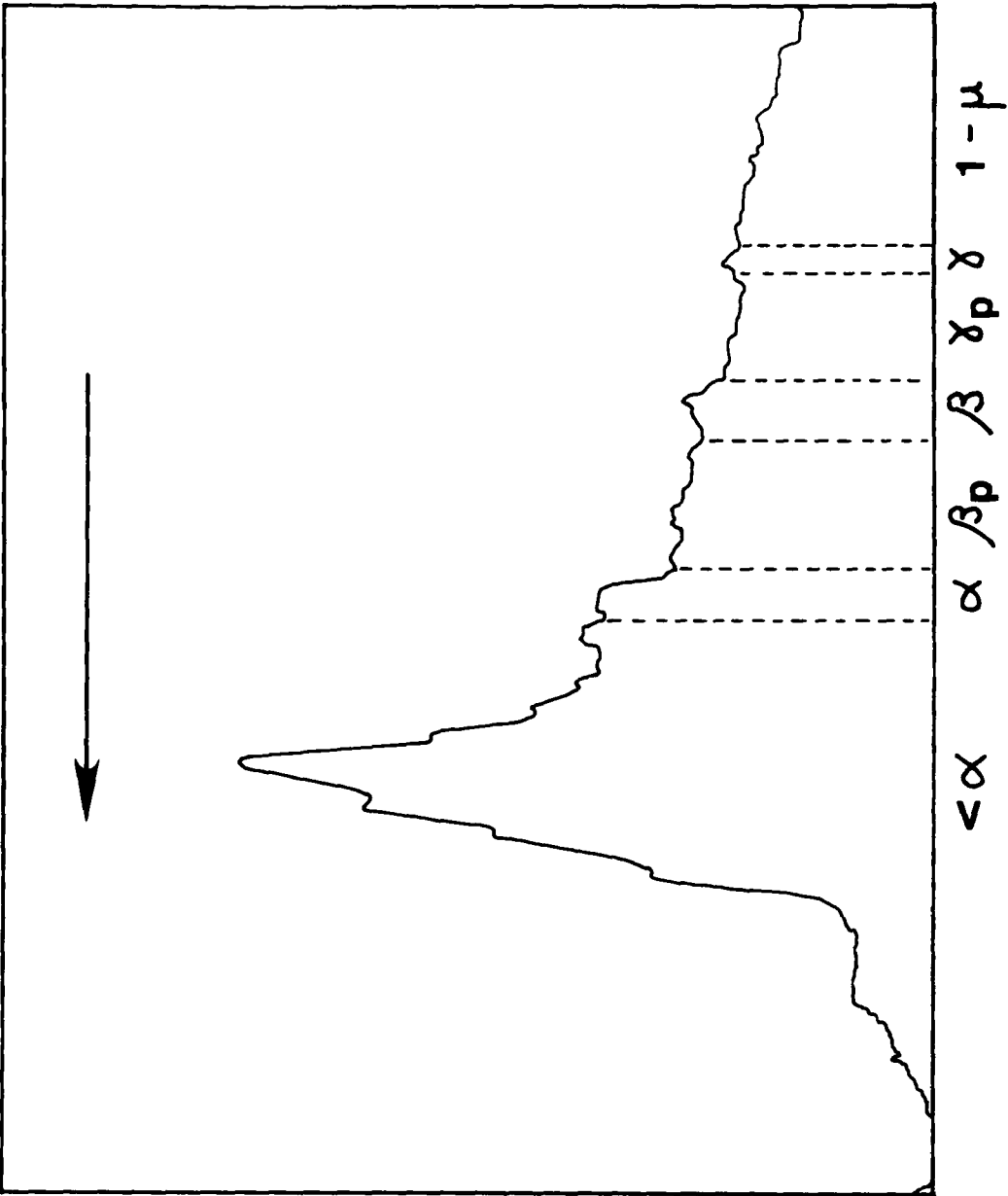
TABLE 3.7 The Reproducibility of PAGE Results for
Limed Ossein Gelatin DFG/Stoess 2766

FIGURE 3.8

Densitogram of the Electrophoretic Separation of an Acid
Ossein Gelatin on 2.5% Polyacrylamide Gel

Gelatin : Croda 472

➡ : Direction of Electrophoretic Separation



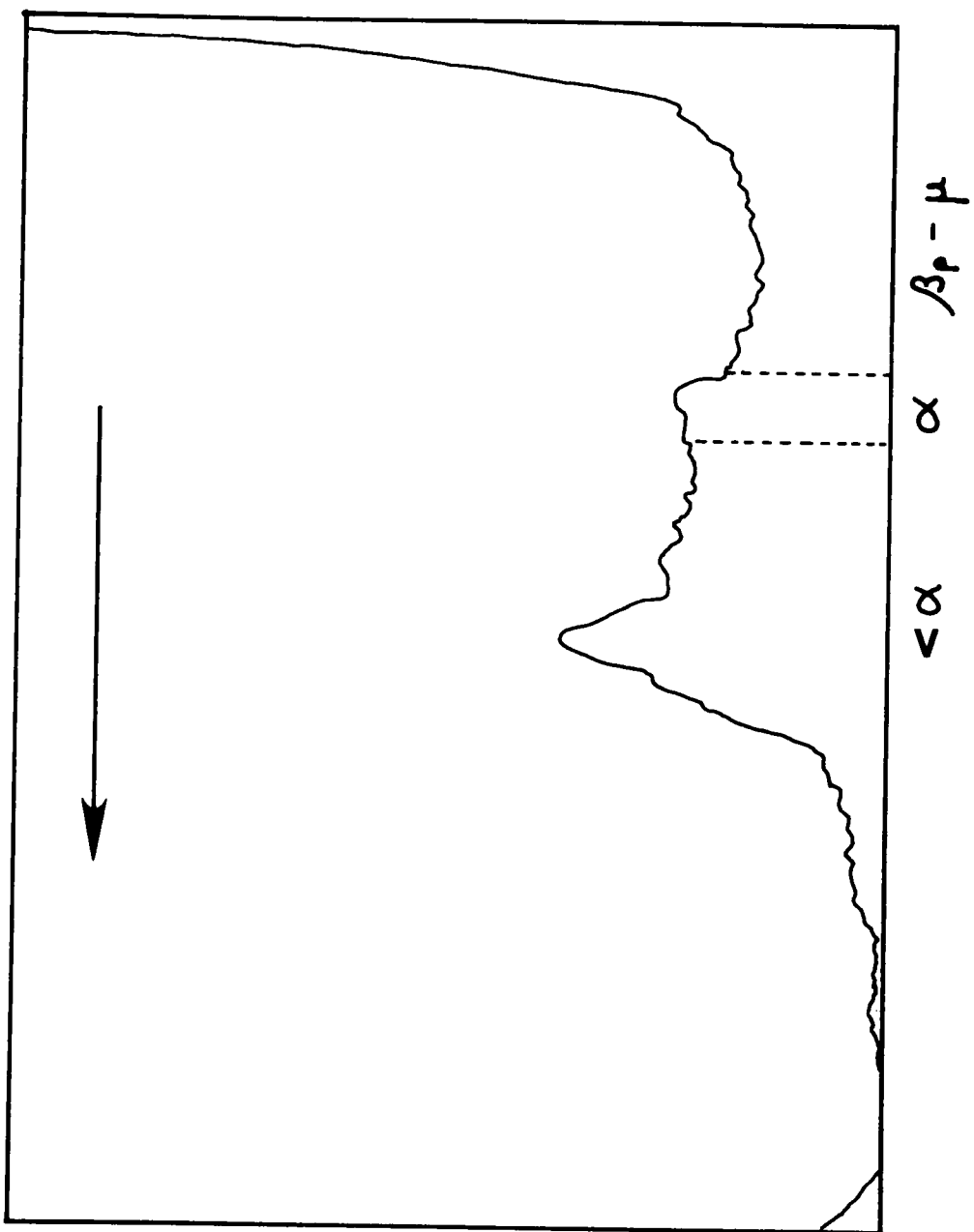
OD

FIGURE 3.9

Densitogram of the Electrophoretic Separation of an Acid
Ossein Gelatin on 5% Polyacrylamide Gel

Gelatin : Croda 472

➡ : Direction of Electrophoretic migration



o D

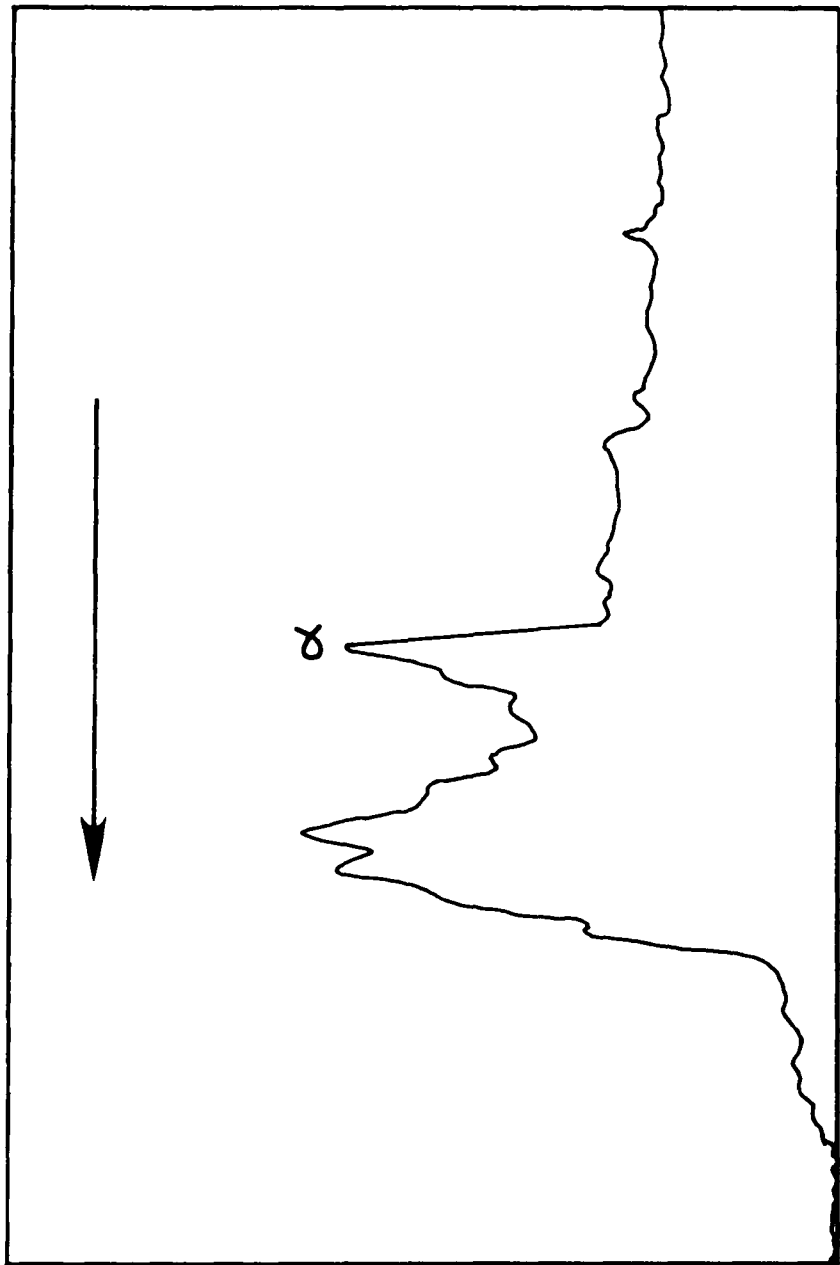
FIGURE 3.10

Densitogram of the Electrophoretic Separation of a 2:1
Acid : Limed Ossein Gelatin Mixture on 2.5% Polyacrylamide
Gel

Gelatin : Acid Ossein Croda 472, Limed Ossein Batch 1

➡ : Direction of Electrophoretic Migration

OD



COMPONENTS	>g	g + g _p	a	<α
<u>Composition (%)</u>				
Tomka (19)	23.1	19.0	5.5	52.5
This work - RUN 1	25.5	17.7	5.7	51.1
RUN 2	25.4	20.2	5.8	48.6
RUN 3	25.2	18.2	6.3	51.8
<u>Range of Variation (%)</u>				
	0.3	2.5	0.6	3.2

TABLE 3.11 Reproducibility of PAGE Results for Acid
Ossein Gelatin Croda 472 and Comparison with
Results obtained by Tomka (85)

FIGURE 3.12

Densitogram of the Electrophoretic Separation of an Acid
Pigskin Gelatin on 2,5% Polyacrylamide Gel

Gelatin : Batch 9



: Direction of Electrophoretic Migration

OD

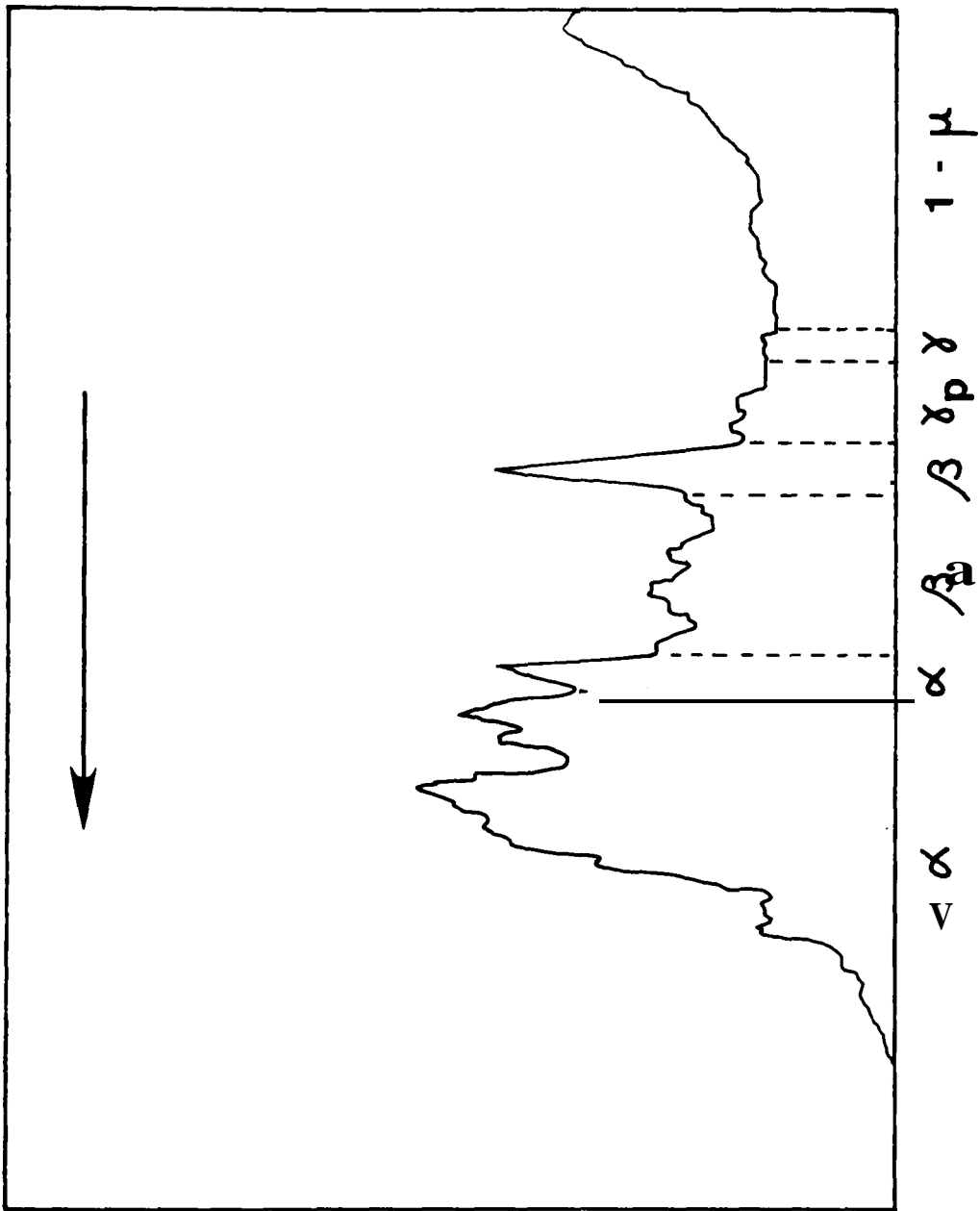


FIGURE 3,13

Densitogram of the Electrophoretic Separation of an Acid
Pigskin Gelatin on 5% Polyacrylamide Gel

Gelatin : Batch 9



: Direction of Electrophoretic Migration

OD

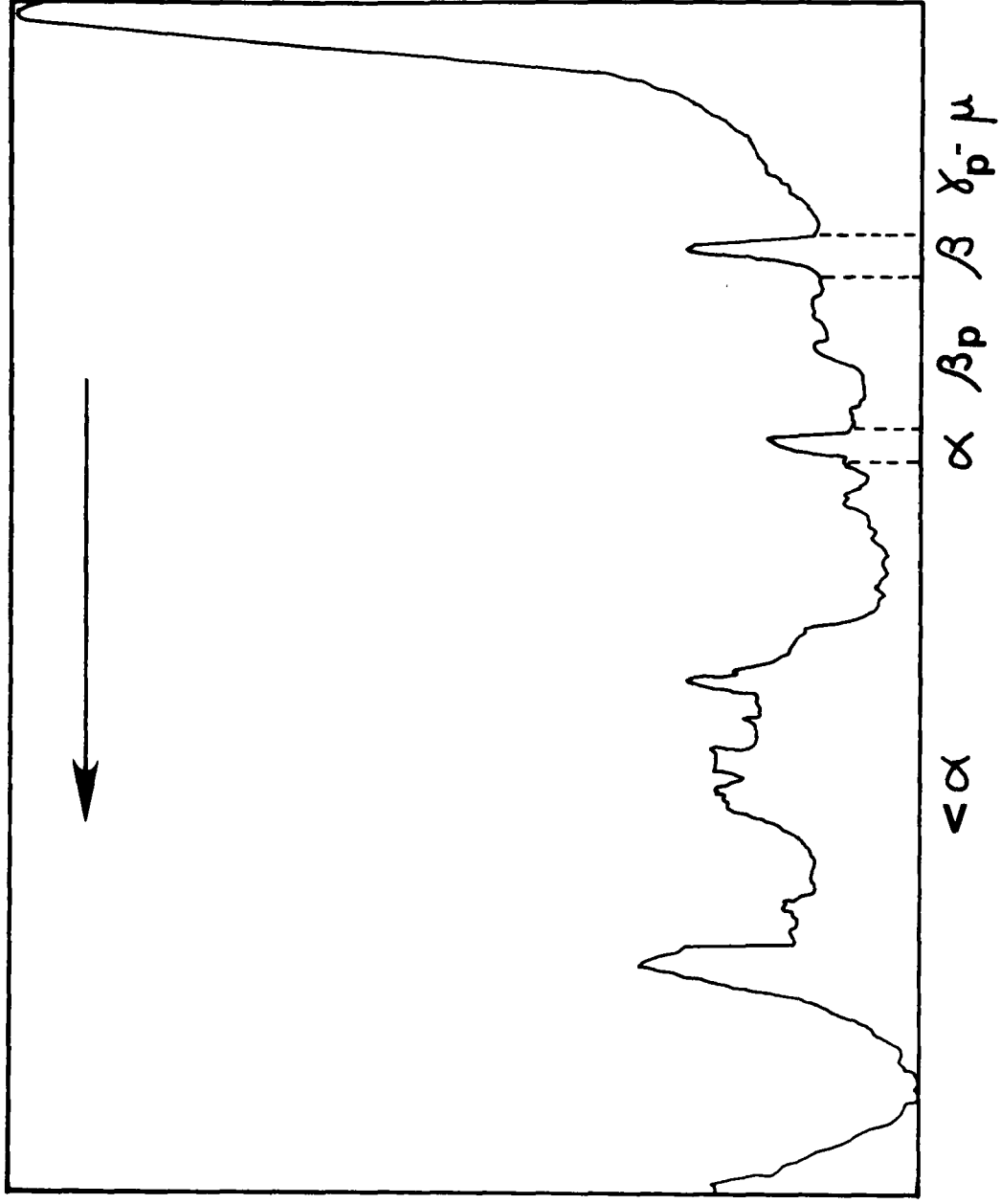


FIGURE 3,14

Densitogram of the Electrophoretic Separation of the
60 Bloom Alkali Hide Gelatin on 2.5%Polyacrylamide Gel

Gelatin : Batch 10

➡ : Direction of Electrophoretic Migration

o D

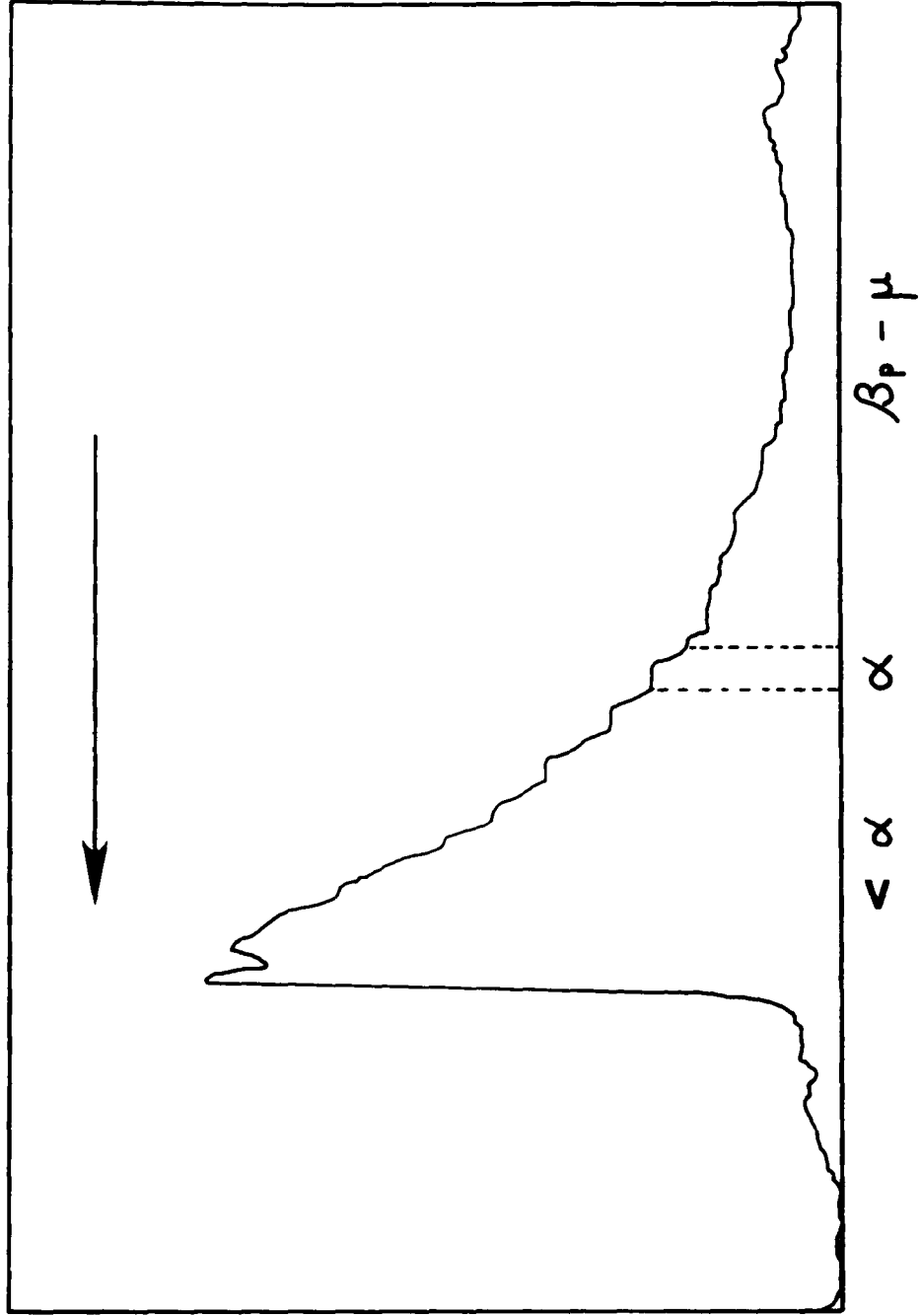
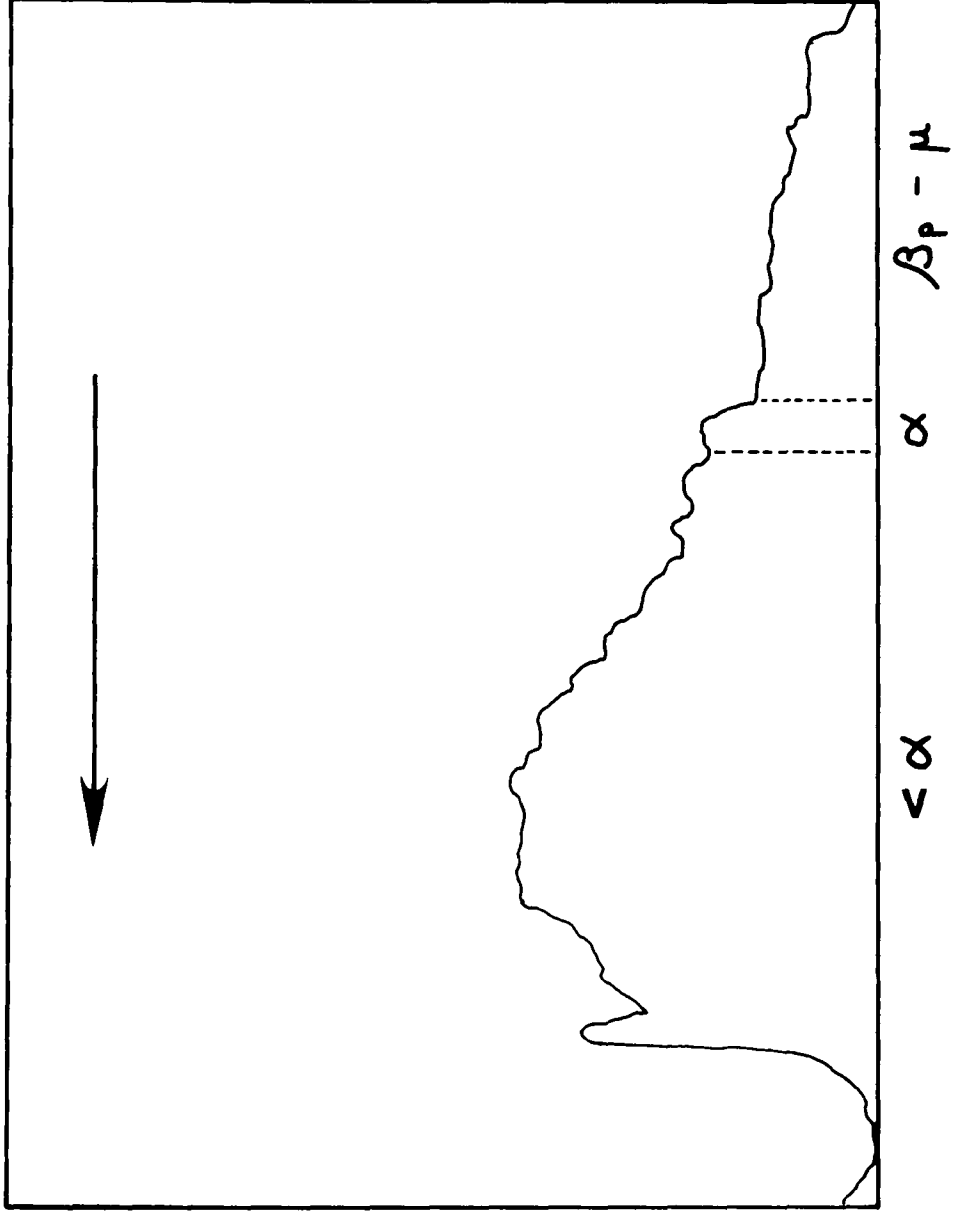


FIGURE 3,15

Densitogram of the Electrophoretic Separation of the
60 Bloom Alkali Hide Gelatin on 5% Polyacrylamide Gel

Gelatin : Batch 10

➡ : Direction of Electrophoretic Separation



OD

GELATIN TYPE	BATCH NO.	CONTENT OF COMPONENTS (%)				
		>g	$\beta + \beta_p$	α	A	<A
LIMED OSSEIN (Good)	[1	21.3	15.8	20.3	4.5	38.1
	2	24.3	14.9	27.3	6.8	26.7
	3	21.8	18.6	21.1	3.6	34.9
LIMED OSSEIN (Poor)	[4	22.2	15.7	23.6	3.9	34.6
	5	28.7	16.0	23.2	3.1	29.1
	6	21.6	17.1	23.7	5.6	32.1
ACID OSSEIN	[7	28.6	15.1	4.4		52.0
	8	22.6	17.4	6.0		55.5
ACID PIGSKIN	/ 9	31.1	22.1	5.6		41.1
ALKALI HIDE 60 BLOOM	[10	10.1	12.6	4.0		73.3

TABLE 3.16 The Molecular Composition by PAGE of
Gelatins used in this Work

The composition of all the gelatins used in this work was determined by PAGE. A summary of the results is given in Table 3.16 and the densitograms are contained in Appendix 1.

3.1.5 Discussion

The MMD of a gelatin is dependent upon the source tissue and the treatment it receives during manufacture, in **particular, the** pretreatment and extraction stages.

Mature tissues such as ox-hide and ossein are more heavily cross-linked and subsequently require more severe treatment to produce good yields of gelatin than immature tissues such as pigskin (9,87-89). Batch variations may be introduced by different tissue ages and uncontrolled degradation (microbial or by drying) of raw materials prior to manufacture (4).

LIMED OSSEIN GELATINS

The densitograms (Figures 3.2, 3.3 and Appendix 1) reveal a large number of α chains, with discernable peaks for the g and γ components, superimposed upon a broad spectrum of oligomeric and fragmented molecules from μ -gel to lower peptides. Lime pretreatment is considered to selectively cleave specific bonds on the collagen molecule, in particular the covalent cross-links (3). Upon extraction, this results in the release of many intact α -chains and smaller numbers of intact g, γ and higher oligomers containing the surviving cross-links (90). The underlying

continuous spectrum of oligomers and fragments is formed partially by the limited peptide bond cleavage that occurs during pretreatment and partially by the increasingly severe conditions of successive extractions, that together release both higher and lower molecular mass material.

Of the limed ossein gelatins used in this **work**, Batch 2 possessed the largest α content and a low proportion of lower peptides. Batch 5 also has a smaller lower peptide content than the others, but has larger amounts of high molecular weight components. There appears no clear distinction, on the basis of the major components of the MMD, between 'good' and 'poor' limed ossein batches for capsule production.

ACID OSSEIN GELATINS

In acid pretreatment non-specific peptide bond cleavage predominates (89). Crosslinks are preserved, leading to more compact molecules that consist of lateral **aggregates** of chain fragments held together by cross-links (71,91). The non-selective nature of the peptide bond disruption is revealed in the densitograms which show a more uniform degree of molecular comminution than limed ossein, without a prominent α peak and with more lower peptides. The comments in the previous paragraph pertaining to extraction are also applicable here.

There is little difference between the MMD of the two acid ossein gelatins used in this work. The higher γ and lower $g + gp$ fraction of batch 7 may be explained in the uncertainty of ascertaining the position of the g peak.

ACID PIGSKIN GELATINS

The most notable features of the densitogram are the large $g : a$ peak ratio and the broad initial peak of very high molecular mass material apparent on the 2.5% gel trace. Pigskins as supplied, are a young tissue with little covalent crosslinking and they therefore require less rigorous treatment than mature ossein for good **yields**. a -chains are more easily extracted **intact, but** as most crosslinks are preserved by acid pretreatment, they appear as oligomeric species. The lower extraction temperature (9) and absence of a demineralization stage curtails the peptide bond hydrolysis at these stages and a greater proportion of high molecular weight material survives into the final gelatin. This is apparent from the determined composition (Table 3.16) where over 50% of the gelatin is of molecular mass greater than the a fraction.

ALKALI HIDE (60 BLOOM) GELATIN

The densitograms show this gelatin to possess a large proportion of lower peptides and the lack of an initial peak on the 5% gel trace indicates that little material of high molecular mass is present. In these respects and with a lack of recognizable peaks, the trace is consistent with that published for a highly degraded limed ossein gelatin with low gel strength and long setting time (93).

3.2 OTHER PHYSICO-CHEMICAL PROPERTIES

3.2.1 Manufacturers Analytical Data

The results of routine quality control tests carried out by the capsule manufacturer are listed in Table 3.17. The methods used are those of BS757:1975. The gelatins are within **specification** for the high quality of raw material required for capsule production. Ash contents are particularly low in batches 1, 7, 8 and 9 which have been deionized during manufacture (112). pH and turbidity values were determined in this laboratory. Turbidity was measured using an EEL nephelometer with Unigalvo Head Type 20. (Evans Electro^eselnium Ltd., Hailstead, Essex, UK). 25% ^w/w solutions at 50°C were compared with a tinted standard prepared at 25% turbidity of the ground perspex standard. Results are expressed as percentage turbidity relative to the perspex standard. For limed gelatins at **non-isoelectric** pH, excess turbidity can indicate the presence of suspended foreign matter (113). Acid processed gelatins show a broad pH range of turbidity around the isoelectric point (91,96) and this test is less reliable.

3.2.2 The Determination of Isoionic Point

The isoionic point (**pI**) is the pH at which a **protein** molecule possesses equal numbers of negative and positive charges, in the absence of ions other than H^+ and OH^- (94). It may be measured as the pH of a deionized protein solution (95).

GELATIN TYPE		LIME O SSEIN GOLO CA SULES		LIMED OSSEIN GOLO CAPSULES		ACT ⁿ OSSEIN		ACT ⁿ ALKALIE WIGKIN ⁿ T ⁿ	
		1	2	3	4	5	6	7	8
WAT ⁿ		1	2	3	4	5	6	7	8
WAT ⁿ (g) 8.7%		24.8	24.4	28.8	24.7	23.2	24.2	25.9	24.0
Viscosity (at 12.5% at 80°C)		190	218	218	207	200	195	198	185
Viscosity (at 12.5% at 80°C)		20.0	18.9	14.8	18.9	17.8	25.2	18.0	14.7
WAT ⁿ (g) 8.7%		24.0	4.0	2.0	3.0	3.0	8.8	19.0	8.0
WAT ⁿ (g) 8.7%		0.20	0.88	0.57	0.80	0.77	0.88	0.10	0.08
WAT ⁿ (1.5% solution)		5.58	5.88	5.88	5.74	5.79	5.87	5.88	5.88
WAT ⁿ (1.5% solution)		21.4	7.5	18.7	11.8	22.3	14.1	58.1	19.8
WAT ⁿ (1.5% solution)		21.4	7.5	18.7	11.8	22.3	14.1	58.1	19.8

WATⁿ 8.17 Analytical data for Gelatin as supplied to the Capsule Manufacturer

(Produced with permission: E. L. Lilly Ltd., Washington, D.C.)
: E. L. Lilly Ltd., Washington, D.C.

100 ml of freshly-prepared 2% gelatin solution was passed through a column (40 cm x 4 cm) of Amberlite MB-3⁺ mixed-bed ion exchange resin maintained at 60°C (97). The first 50 ml of eluate was discarded and subsequent portions recycled until consistent readings of pH were obtained. A Corning Model 113 pH meter with a glass electrode was used, freshly calibrated at pH 4 and 7. Limed gelatins exhibit a sharp turbidity maximum at the **pI** and the appearance of turbidity on cooling provided a useful check that the isoionic state had been achieved. For acid pretreated gelatin this check is less reliable (91,96). Isoionic points are shown in Table 3.18.

The **pI** of collagen has been estimated to lie between 9.0 - 9.4 (15). Pretreatment of raw materials causes deamidation of asparagine and glutamine side chains with a consequent increase in the number of ionized carboxyl groups (98,99). The loss of amide nitrogen and lowering of **pI** have been quantitatively related (100,101). Amide conversion occurs extensively (**pI** = 4.8 - 5.0) during lime pretreatment but much less so during acid pretreatment (**pI** = 6.0 - 9.3) where the extent depends on the severity of manufacturing conditions. Acid ossein gelatins have lower **pI** values than acid pigskin gelatins because of demineralization (102), and have a broader distribution of charged species than limed ossein gelatins because of unequal deamidation of molecules during manufacture (103).

+ BDH Chemicals, Poole, Dorset, U.K.

GELATIN TYPE	BATCH	pI
Limed Osseins (Good)	1	4.94
	2	4.88
	3	4.90
Limed Osseins (Poor)	4	4.82
	5	4.90
	6	4.90
Acid Osseins	7	6.85
	8	6.62
Acid Pigskin	9	8.78
AlkaliHide 60 Bloom	10	5.55

TABLE 3.18 The Isoionic Points of Gelatins (pI)

3.2.3 The Detection of Protein Impurities by Ultraviolet Spectroscopy.

Protein solutions exhibit intense absorbance in the ultraviolet below 250 nm. due to peptide bonds, and between 250 - 300 nm less intense peaks are observable from the aromatic amino acids **tyrosine,phenylanilineand** tryptophan (86), Collagen is particularly poor in aromatic amino acids and a highly purified preparation shows low and unremarkable absorbance in this region (104), The spectrum over this region can therefore be used to indicate the degree of contamination by other proteins present as impurities (105),

Two observations are pertinent to the application of this procedure to gelatins.

(i) Limed gelatins possess intrinsically lower tyrosine levels than acid processed gelatins, because of the loss of tyrosine-rich telopeptides during liming (15). Tyrosine peak measurements at relatively high concentrations should therefore be a suitable test for tyrosine-rich impurities in limed gelatins.

(ii) Nucleotide **bases,which** are present to an extent dependent on source tissue and manufacturing process, complicate the UV spectrum in this region. Fortunately, ionisation of the tyrosyl hydroxyl group in alkaline solution shifts the tyrosine peak maximum from 275 nm to 292 nm, away from almost all interfering nucleotide base maxima (86,106).

Cobett has shown that the shifted tyrosine peak in gelatin is coincident with that of pure tyrosine above 300 nm (111) and chose 304 nm for quantitative estimations. Various workers (108-110) have isolated in varying amounts, a mucoprotein-derived impurity from gelatin which had a tyrosine content a magnitude greater than that of the parent gelatin. The impurity content was related only to the efficiency rather than the mode of manufacture of the gelatin.

1% gelatin solutions in water and 1MNaOH were scanned between 240-320 nm using a Perkin-Elmer*554 UV/visible spectrophotometer with quartz cells, and the appropriate solvent as blank. Figure 3.19 shows spectra from each gelatin type. They exhibit weak peaks at the same wavelengths but these differ in intensity. The relatively low absorbance of the limed ossein gelatin may be ascribed to the loss of tyrosine and purine bases that occur on liming. The spectral shift in alkaline solution is shown in Figure 3.20. The only remaining interfering nucleotide base is uracil (γ max = 284 nm (48)) which accounts for the broadening of the tyrosine peak at lower wavelengths. Table 3.21 lists the specific absorbance coefficients at 304 nm (corrected for ash and moisture) of both 'good' and 'poor' limed ossein gelatins. Amongst the limited number of limed ossein gelatins available there appears no distinct relation between tyrosine content and suitability

* Perkin-Elmer Ltd., Beaconsfield, Bucks. HP9 1QA, U.K.

FIGURE 3.19

The Ultraviolet Spectrum of Different Gelatin Types
in the Range 240 - 300 nm

E $\frac{1\%}{1\text{cm}}$ - Specific **Absorbtion** Coefficient (94)

- | | | | |
|---|---|--------------------------------|----------|
| A | - | Alkali Hide (60 Bloom) gelatin | Batch 10 |
| B | - | Acid Ossein gelatin | Batch 8 |
| C | - | Acid Pigskin gelatin | Batch 9 |
| D | - | Limed Ossein gelatin | Batch 3 |

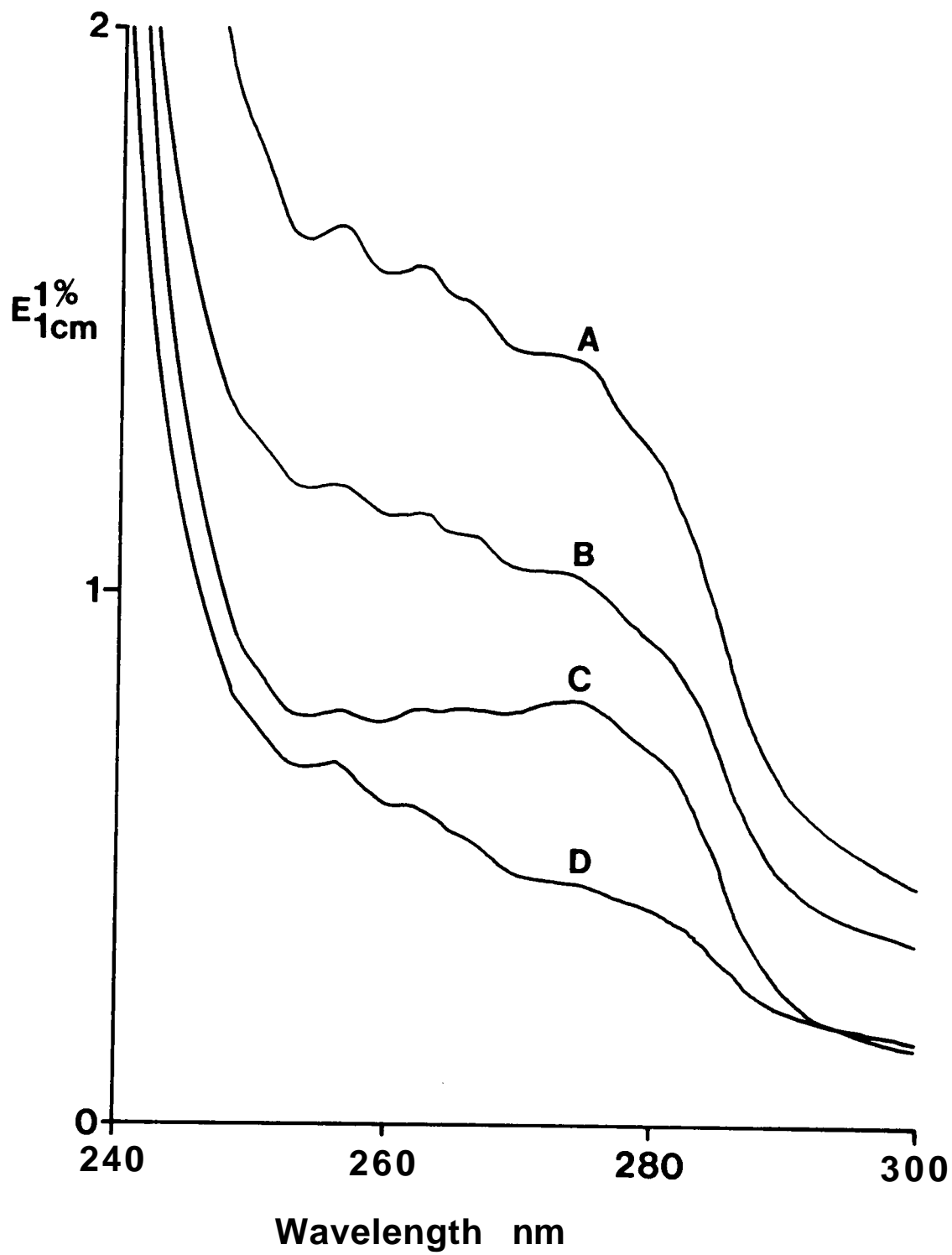
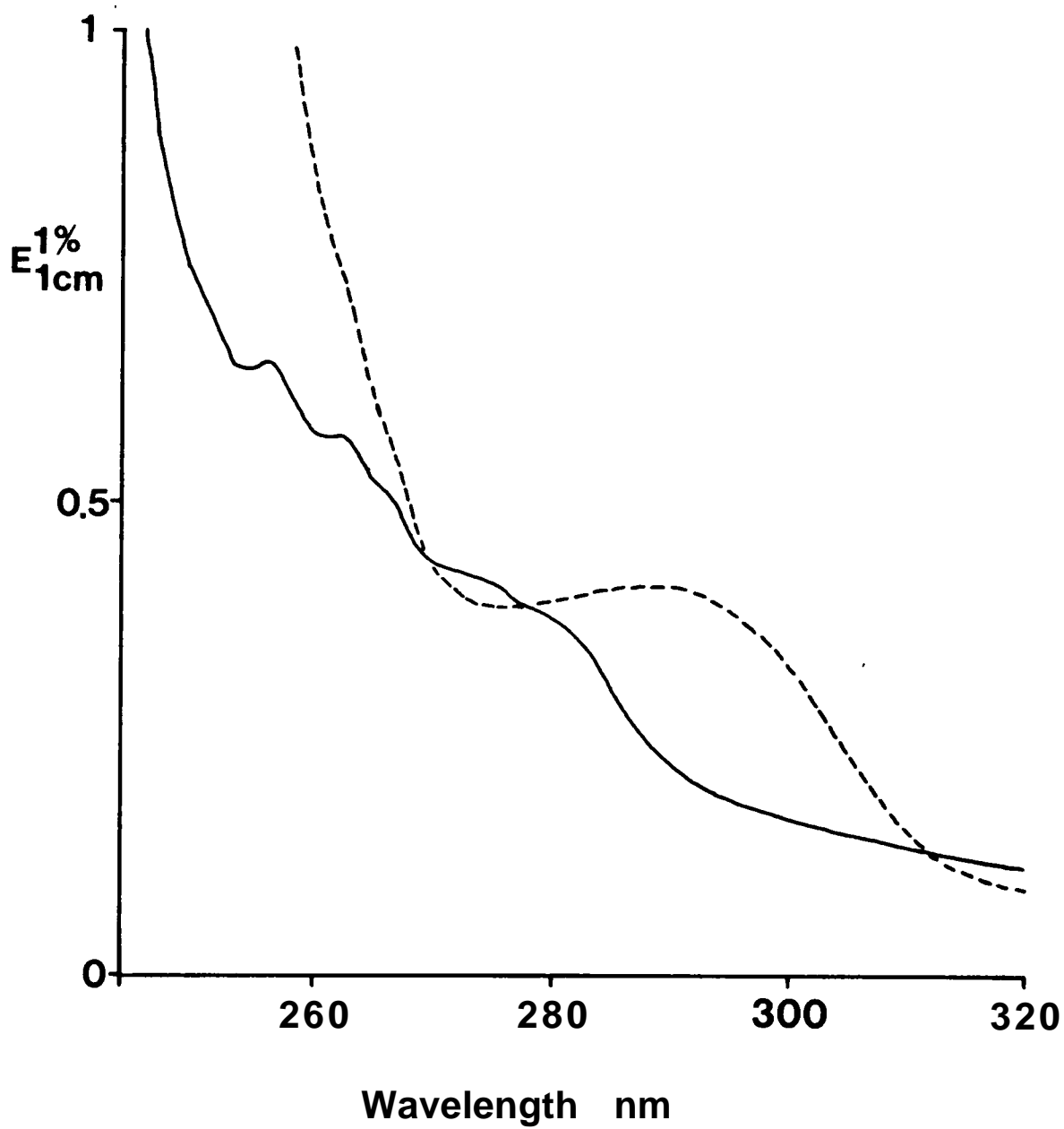


FIGURE 3.20

The Tyrosine Peak Shift in the Ultraviolet Spectrum of
Gelatin in Alkaline Solution

$E_{\frac{1\%}{1\text{cm}}}$ - Specific Absorbance Coefficient

_____ Solution of Gelatin Batch 3 in distilled water
----- Solution of Gelatin Batch 3 in 1M NaOH



GELATIN TYPE	BATCH	$E_{1\text{cm}}^{1\%}$
Limed Osseins (Good)	[1	0.358
	2	0.374
	[3	0.405
Limed Osseins (Poor)	[4	0.391
	5	0.401
	[6	0.394
Acid Osseins	[7	0.741
	[8	0.752
Acid Pigskin	[9	0.745
Alkali Hide	[10	0.806

TABLE 3.21 Specific Absorbance Coefficients ($E_{1\text{cm}}^{1\%}$)
at 304 nm

for capsule manufacture. The alkali processed hide gelatin appears to possess a high tyrosine content.

3.3 THE DEGRADATION OF GELATIN SOLUTIONS PREPARED FOR FILM SPREADING

In the capsule manufacturing plant 30% ^w/w gelatin solutions are prepared and allowed to degas at 60°C for 10 hours. This time-scale is inconvenient for laboratory work and the film spreading technique necessitates the use of a less concentrated solution. In the forthcoming chapters, films have been prepared from 25% ^w/w gelatin solutions that have been allowed to degas at 50°C for 18 hours. This experiment compares by viscometry, the degradation undergone by gelatin solutions prepared by the industrial and laboratory methods.

25 and 30% ^w/w solutions in sealed containers were prepared by swelling 4 hours then stirring and melting at 60°C for 15 minutes. 30% solutions were allowed to stand at 60 ± 1°C for 10 hours and 25% solutions at 50 ± 1°C for 18 hours to degrade. Dynamic viscosity measurements at 60°C were performed on solutions diluted to 6.7% ^w/w using a U-tube viscometer type C. The viscometer constant at 60°C was determined using Dow-Corning 200/20 cs Silicone fluid.

The dynamic viscosity η was calculated from the relationship:

$$\eta = \frac{q \cdot t}{k}$$

η : dynamic viscosity ($\text{Kg m}^{-2} \cdot \text{s}^{-1}$)
 q : density of solution (Kg m^{-3})
 k : viscometer constant
 $(2.727 \times 10^7 \text{ m}^{-2} \cdot \text{s}^2)$
 t : time (s)

and the percentage viscosity drop by

$$D_{\eta} = \frac{\eta_0 - \eta}{\eta_0} \times 100$$

- D_{η} : percentage viscosity drop
 η_0 : dynamic viscosity of an undegraded gelatin solution of 6.7% w/w concentration
 η : dynamic viscosity of a degraded gelatin solution of 6.7% w/w concentration

η_0 was determined on undegraded 6.7% w/w solutions using the same viscometer.

Percentage viscosity drop values are listed in Table 3.22. Most gelatins undergo slightly less viscosity degradation under laboratory preparative conditions than industrial. The viscosity degradation of a gelatin is a function of pH, temperature and proteolytic enzyme content (3,51) and may be related to an increase in lower peptide content (114) and a decrease in higher molecular weight species (86),

GELATIN TYPE	BATCH	PERCENTAGE VISCOSITY DROP %	
		A	B
Limed Osseins (Good)	[1	5,9	5.1
	2	4,0	2.9
	3	6,5	3.2
Limed Osseins (Poor)	[4	4.2	2.6
	5	9,9	8.9
	6	8,3	6.6
Acid Osseins	[7	5,8	5.0
	8	4,0	4.2
Acid Pigskin	[9	7.4	8.0
Hide (60 Bloom)	[10	4.5	2.6

A : Industrial conditions 30% 60°C 10 hours

B : Laboratory conditions 25% 50°C 18 hours

TABLE 3.22 The Degradation of gelatin solutions under
Laboratory and Industrial preparative
Conditions

CHAPTER IV

THE DRYING OF GELATIN FILMS

4.1 INTRODUCTION

The drying of a slab of wet solid into a moving air stream under constant conditions is classically represented by the drying rate curve shown in **Fig.4.1**, which may be subdivided into several distinct drying periods (156, 157):

INITIAL EQUILIBRATION (A-B)

A short period of temperature equilibration as the drying surface cools to the wet-bulb temperature (WBT).

CONSTANT RATE PERIOD (B-C)

Drying proceeds at a constant rate from a thin continuous film of water which covers the surface of the material and is maintained by diffusional or capillary transport from the interior. Heat input balances heat lost by vaporization and the surface temperature remains constant at the WBT.

The rate of drying is controlled by the rate of vapour diffusion through the surface stationary air layer.

FIRST FALLING RATE PERIOD (C-D)

Water transport is insufficient to maintain the continuous surface film. Dry spots appear and grow and the drying rate falls steadily. The onset of this period (C) is termed the critical moisture content (X_{Cr}).

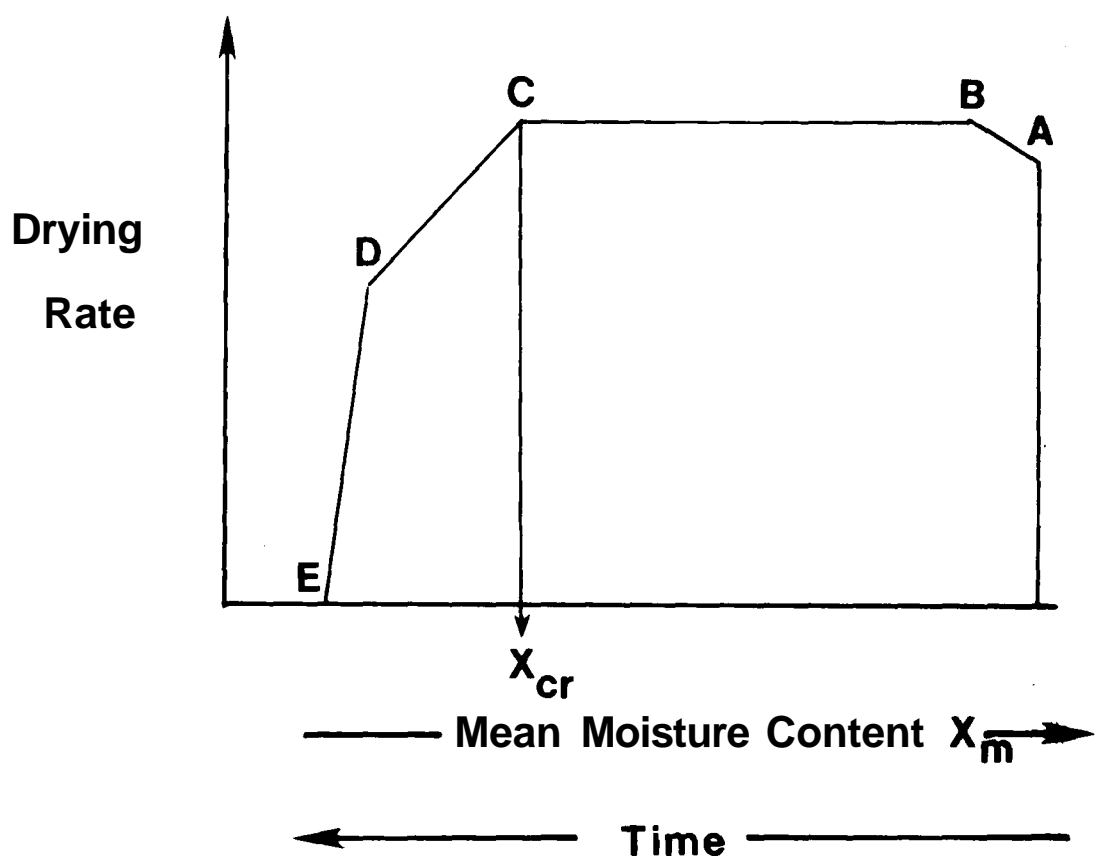
SECOND FALLING RATE PERIOD (D-E)

D marks the secondary critical point (tertiary moisture content) at which the surface film has completely evaporated.

FIGURE 4.1

An Idealised Drying Rate Curve

x_{Cr} is the critical moisture content



The drying rate is controlled by the rate of internal water transport to the surface and decreases more rapidly than in the first falling rate period. Drying time is thickness **dependent**.

EQUILIBRIUM MOISTURE CONTENT (E)

The equilibrium moisture content (EMC) represents the water retained by the material under given conditions. The drying rate is zero and further moisture removal may only be accomplished by altering drying conditions. The amount of removable water (i.e. that above the EMC) within a wet solid prior to drying, is termed the free moisture content (158).

Materials may be classified as porous or non-porous according to their internal structure and subsequent drying behaviour (157, 159).

- a) Porous solids. (e.g. crystalline and granular materials)

Water is held within a network of pores and capillaries. During **drying, porous** solids exhibit a pronounced constant rate period, short falling-rate periods and low EMC and X_{Cr} . Internal moisture transport is primarily by capillary flow and, during the falling rate periods, the moisture distribution within the solid resembles a receding meniscus.

- b) Non-porous solids (e.g. clays, soaps, starch and other colloidal materials)

Water is more intimately associated with, and may form an integral part of, molecular structure. These materials

exhibit short constant rate and first falling rate periods, prolonged 2nd falling rate periods and comparatively high EMC and X_{Cr} values. Moisture transport is by diffusion and the moisture distribution within the film is qualitatively similar to that predicted by diffusion theory (159). However, complications arise from the variation of diffusion coefficient with moisture content, and surface shrinkage which can markedly reduce water permeability through the dried surface layer (160). Too rapid drying may result in "case hardening" in which a hard dry surface layer forms and makes further drying extremely difficult. Stresses generated by shrinkage may cause warping, cracking and fragmentation of the drying surface (159,164). The tertiary moisture content may be related theoretically to the EMC at 100% R_H and this relationship holds for many colloidal foodstuffs (117).

Gelatin gel belongs to the group of non-porous solids, and the state of water within the gel ranges from 'free' water with almost unrestricted movement to that held rigidly at macromolecular binding sites (122,165). A review of water binding by gelatin is provided in section 5.1,

The slow drying of a thin film (10% w/w) has been shown to exhibit a typical constant rate period and a critical moisture content of $2.4 < X_{Cr} < 3$ (140), Surprisingly, drying rate appears almost independent of film thickness (140,167,168,163)

and Gerhmann and Kast (140) have postulated that the following mechanisms may be responsible:

- 1) The assistance of internal diffusion by hydrostatic pressure generated by shrinkage of surface layers onto the wet core,
- 2) The straining of surface layers(which increases free volume) and stress **orientation, both** of which may alter diffusional resistance.
- 3) Viscoelastic relaxations at the drying surface which, by disturbing the surface moisture **equilibrium, allow** a higher surface moisture level than that provided by diffusional processes alone.

The magnitude of stresses generated by drying is greater for thicker slabs and these effects are enhanced at greater film thicknesses where the drying rate would normally be lower (at a given mean moisture content) if diffusion processes alone prevailed.

When gelatin is rapidly dried, steep internal moisture concentration gradients are set up and there is a tendency to "case-hardening" (7). Faster drying at moisture contents close to the EMC may be achieved by exposure to X-rays (161),

The drying of a shrinking gel is a complex **phenomenon**. As a consequence of shrinkage and straining, internal moisture must traverse a continuous change in molecular structure before reaching the drying surface and the diffusion coefficient

is likely to be moisture dependent, A mathematical model based on the shrinkage of local areas to fill voids left by evaporating solvent has been **presented, but** it only qualitatively fits experimental data (160), In addition, during the drying of a freshly-cast wet capsule shell there is superimposed upon the drying process the concurrent development of internal structure (166),

4,2 SLOW DRYING EXPERIMENTS

4.2.1 Apparatus

Balance

Microforce balance type Mk **IIB** with enclosed head

C.I. Electronics Ltd.
Salisbury. Wilts. UK.

Oven

Incubator-oven hybrid with internal fan

Genlab Ltd.,
Widnes, Cheshire.

Thermometer

"Jenway 2000" Digital Thermometer with **thermocouples**

R.W, Jennings Ltd.,
East Bridgford, Nottingham. UK.

Chart Recorder

Autograph 'S'
Shandon Southern Instruments Ltd.
Camberley, Surrey. UK.

Humidity Sensor

as described in section 2.4

Flow Meter

'Gap' flowmeter

G.A.P. Laton Ltd.
Croydon, Surrey. UK.

Salts for Humidity Control

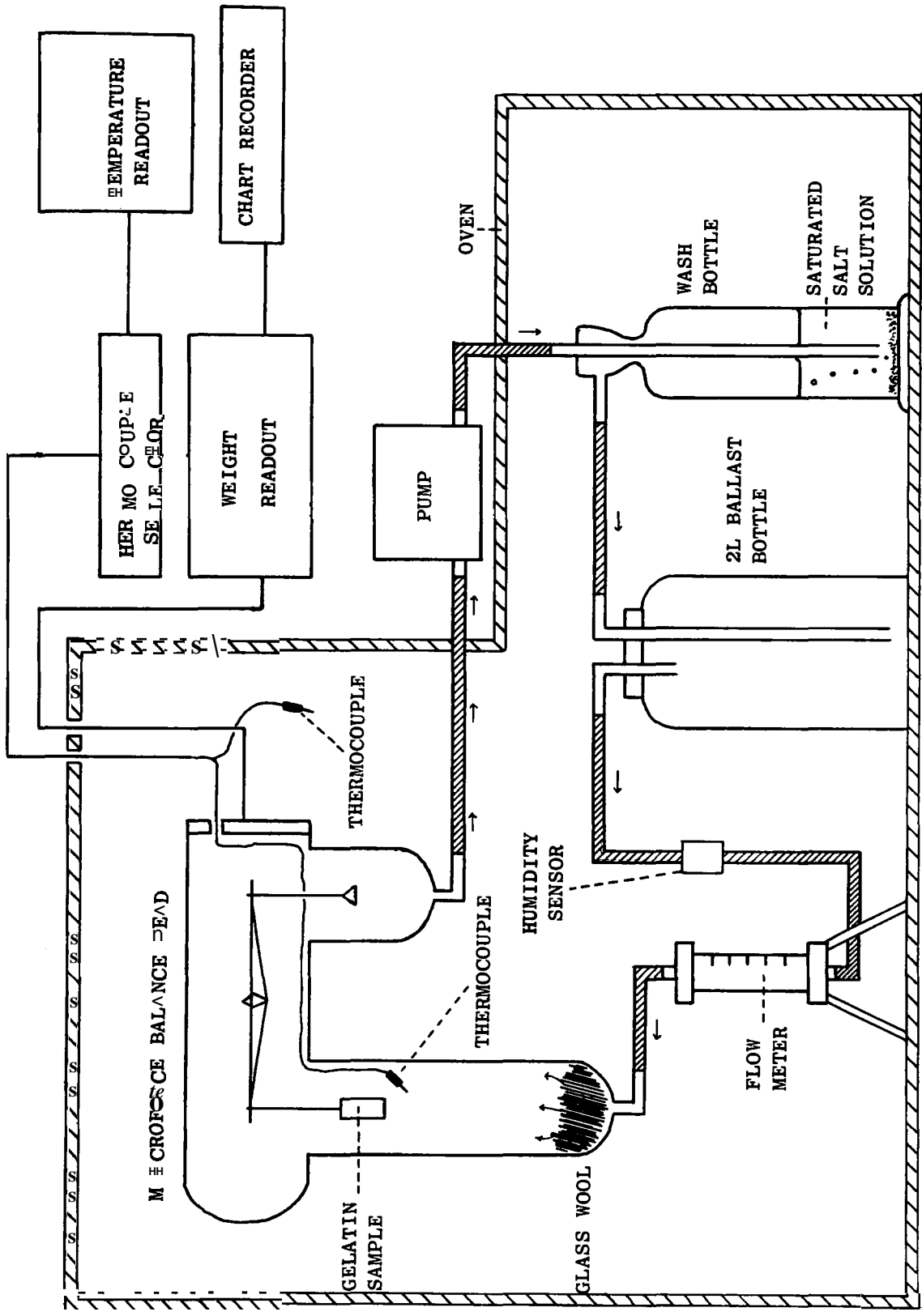
as described in section 2.4.

A diagram of the apparatus is shown in Figure 4.2. The balance head was enclosed within a closed-circuit air-circulating system. A pump provided an airflow of $3,3 \times 10^{-5} \text{ m}^3 \cdot \text{s}^{-1}$ which resulted in an air velocity of $3 \times 10^{-2} \text{ m} \cdot \text{s}^{-1}$ past the sample. Humidity was controlled by the saturated salt solution and monitored by means of the humidity sensor described in section 2.4. Temperature was controlled by enclosing the apparatus within the 'oven' which was a hybrid combining the precise temperature control of an incubator with the rapid re-equilibration characteristics of an oven. Temperature was monitored by means of thermocouples at various points in the oven and the temperature of the airstream before impinging on the sample was carefully controlled to $T \pm 0.2^\circ\text{C}$.

To introduce a sample, it was necessary to break the circuit, and the 2L ballast bottle provided a reservoir of air at the appropriate temperature and humidity to aid rapid re-equilibration. Both temperature and humidity had returned to the appropriate values within 15 minutes and remained stable to $\pm 0.2^\circ\text{C}$ and $\pm 2\% \text{ R}_\text{H}$ throughout the drying run.

FIGURE 4,2

A Diagram of the Apparatus used in the Slow Drying
Experiments



4.2.2 Method

Gelatin films were prepared as described in Section 2.2, Immediately after spreading, a portion of the film surface was overlaid with cover-slips to avoid drying into room conditions. The film was allowed to stand for 12 minutes after which time it was sufficiently firm for manipulation. Pieces of area 2 cm^2 were cut using a template and scalpel and the thickness measured by micrometer, sandwiching the sample between two coverslips. Samples were chosen of the desired thickness $\pm 0.01 \text{ mm}$.

At 18 min. after film formation the sample was hung on the balance arm and dried, the weight being continuously recorded until no further change in weight was evident. The sample was then removed, weighed and subjected to moisture determination (see section 2.3).

These samples were dried from both **sides**. Attempts were made to examine one-sided drying on a restraining support but no satisfactory method was found to attach the film to the backing sufficiently well to prevent peeling.

4.2.3 Results and Discussion

Figure 4.3 shows a typical drying curve. The results were converted to the drying rate : moisture content plot (fig. 4.4) by means of a computer program (Appendix 2) that calculated derivatives of cubic equations fitted to small segments of the curve. The program was verified by plotting

FIGURE 4,3

2

~~The Weight : Time Profile of a 2 cm Piece of Gelatin~~
Film Drying from both sides under Constant Conditions

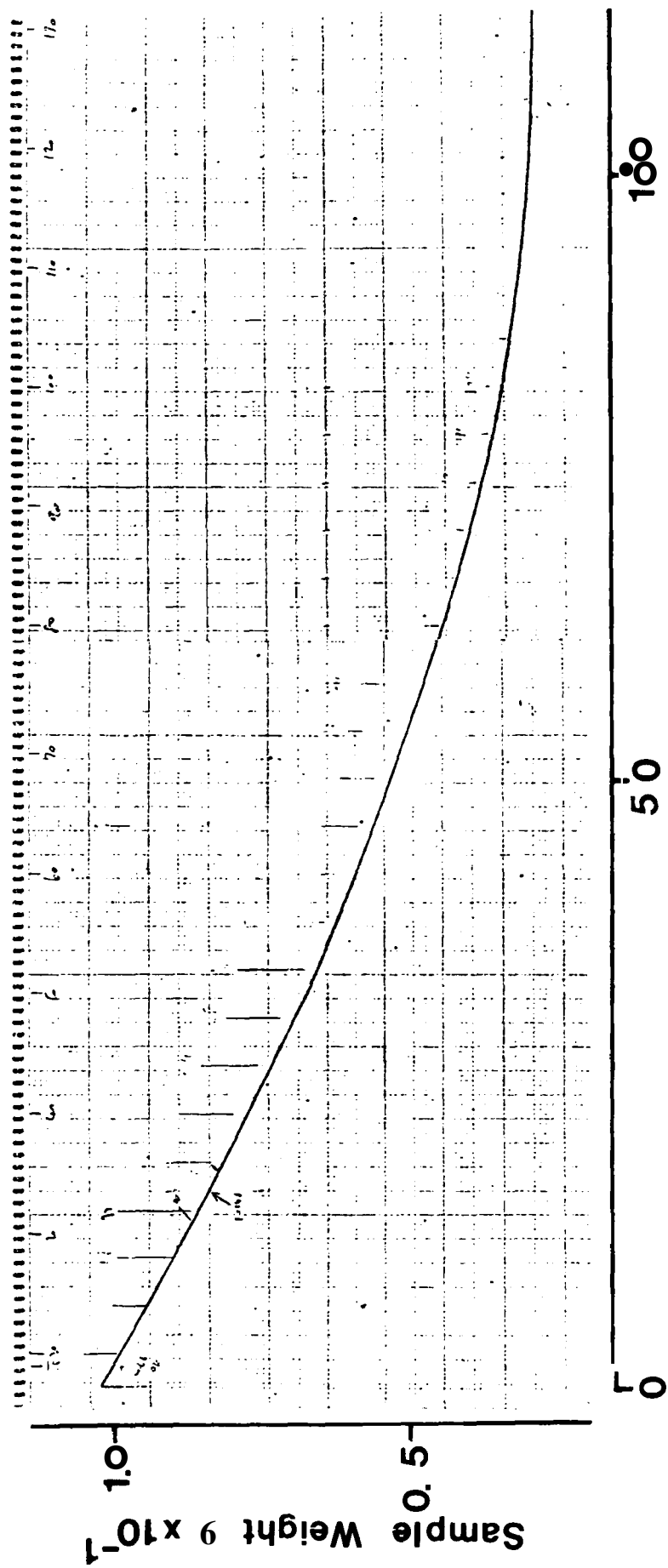
Gelatin Batch 7

Thickness : 0.407 mm

Air velocity : $3 \times 10^{-2} \text{ m s}^{-1}$

Temperature : 25°C

Humidity : 43% R_{H}



Drying Time min.

manually-determined tangents to the drying curve, as shown in Fig. 4.4. The program gave the more precise evaluation of drying rates which are expressed as a rate of water loss for unit original surface area. In concordance with conventional drying experiments (158) the mean moisture content in this chapter alone, is expressed as a dry weight ratio ($\text{kg H}_2\text{O/kg dry gelatin}$) and denoted by X_m . In all other work, moisture contents are expressed as a percentage wet weight ratio (denoted by % moisture content) in line with current practice within the gelatin industry (61).

The drying rate plot (Fig. 4.4) lacks an initial constant rate period. It has been shown (140) that under similar drying conditions the critical moisture content occurs at $2.4 < X_{cr} < 3$ (140) and the drying in these experiments is therefore occurring wholly within the periods of falling rate. A clear secondary critical point is not apparent.

Fig. 4.5 shows the variation of drying rates with film thickness under constant drying conditions. Within the range 0.32 - 0.56 mm, film thickness did not affect drying rates within experimental error, in concordance with the results of previous workers (140, 163). This conclusion assumes uniform shrinkage of surface area. The hypotheses advanced for this unusual result have been detailed in section 4.1. Sample thickness was maintained at 0.40 - 0.41 mm in subsequent experiments, which is the approximate thickness of the wet capsule shell after casting (39).

FIGURE 4.4

Drying Rate against Moisture Content Curve for the Data
from Figure 4.3

X_m = : Mean Moisture Content $\left(\frac{\text{kg H}_2\text{O}}{\text{kg dry gelatin}} \right)$

- Computer calculated drying rates.
- Drying rates determined manually from tangents to the drying curve.

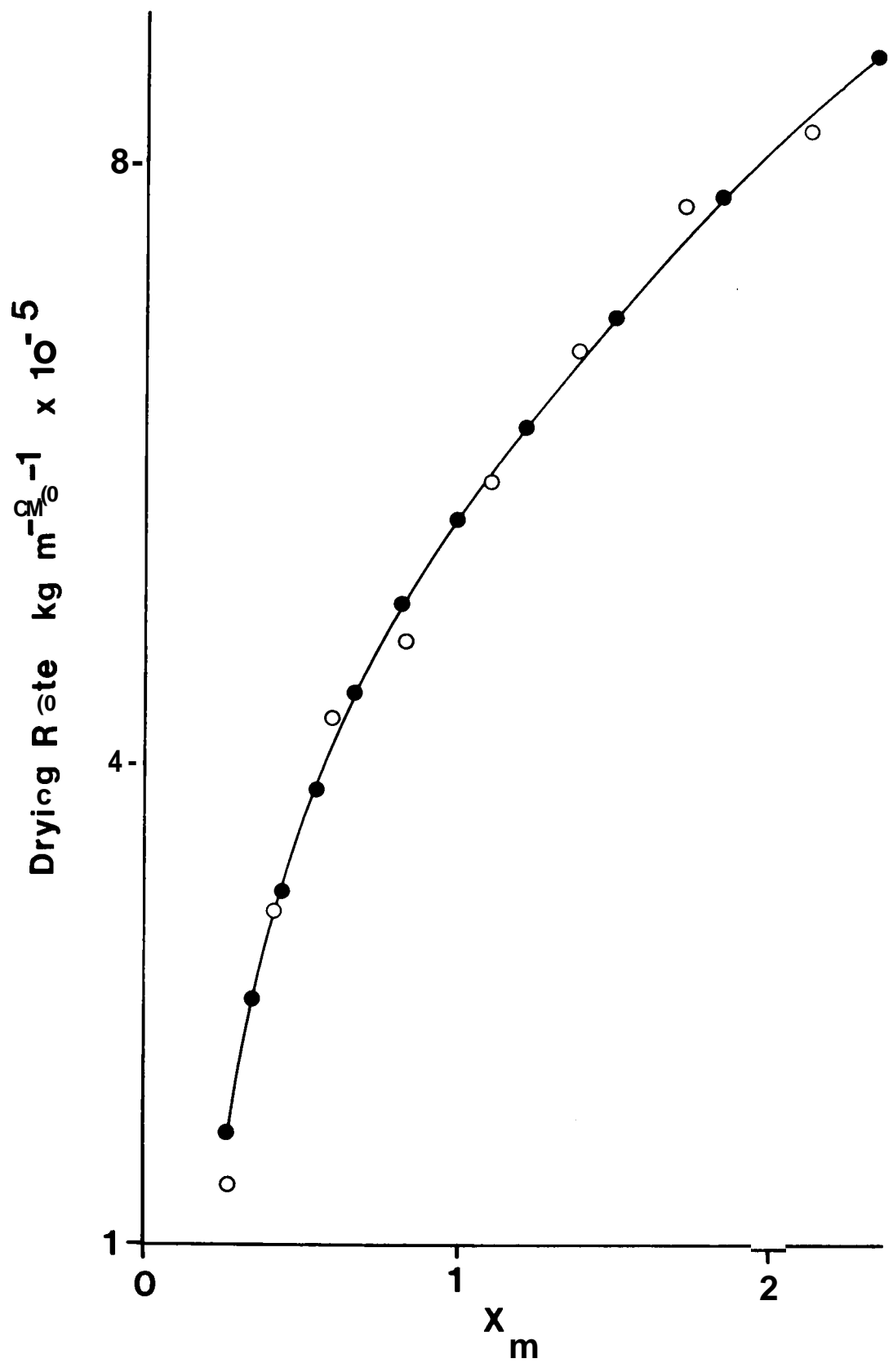
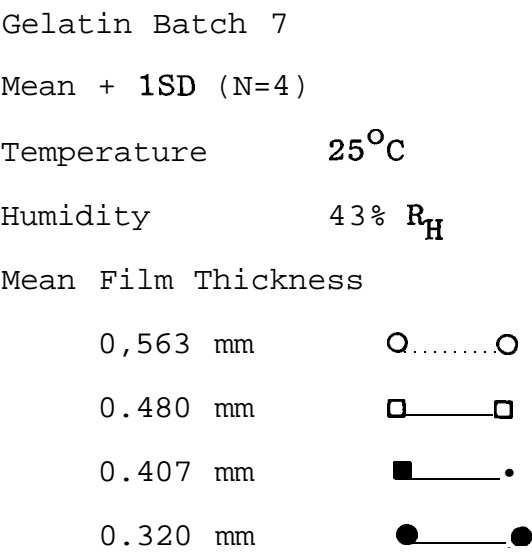
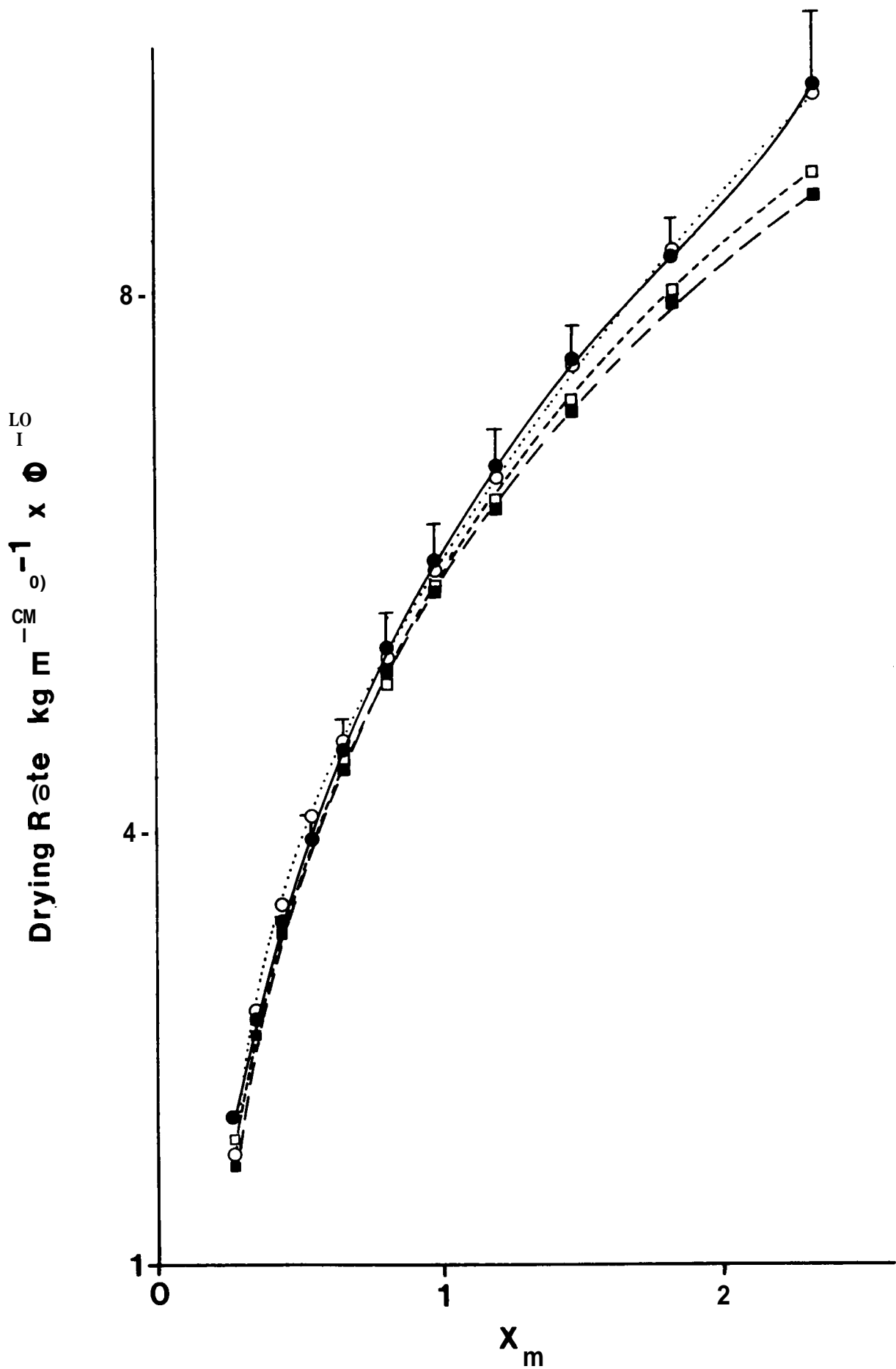


FIGURE 4,5

The Variation of Drying Rate with Film Thickness



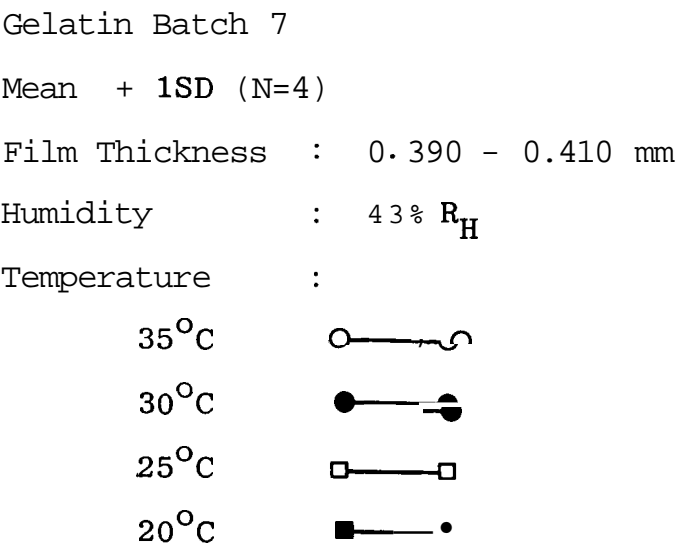


The variation of drying rates with temperature and humidity in this system are shown in Figs. 4.6 and 4.7. For the comparison of gelatins, drying conditions of 43% R_H and 25°C were chosen; close to those used commercially to dry the wet capsule shell. Fig. 4,8 shows the drying rate : moisture content curves for representative samples of different gelatin types. Within experimental error, no differences in drying rates are apparent. As a check on this result the mean drying times between X_m 1.9 to 0.25 were compared. (Above $X_m = 1.9$ reproducibility is poorer ; presumably the sample had not yet fully equilibrated up to this point. Below $X_m = 0.25$ the reproducibility is also poorer due to the sample twisting and disturbing the air flow.) Table 4.9 shows the mean drying times between these moisture contents. Batch 7 may be shown statistically different to batches 8 and 9 (T-test : $0.01 < p < 0.05$), but the overall range of mean drying times is only of the order of 5% (3 min) and it was considered doubtful whether experimental error had been eliminated to this degree in this experiment.

In these experiments the overall drying time was around 80 min. In the industrial situation capsules are dried in 40 min. as a result of higher air velocities. Although slow-drying experiments are useful for determining precise drying rates, it is conceivable that varietal differences may only be apparent at the faster drying rates used commercially, when a degree of case hardening and less

FIGURE 4.6

The Variation of Drying Rate with Temperature



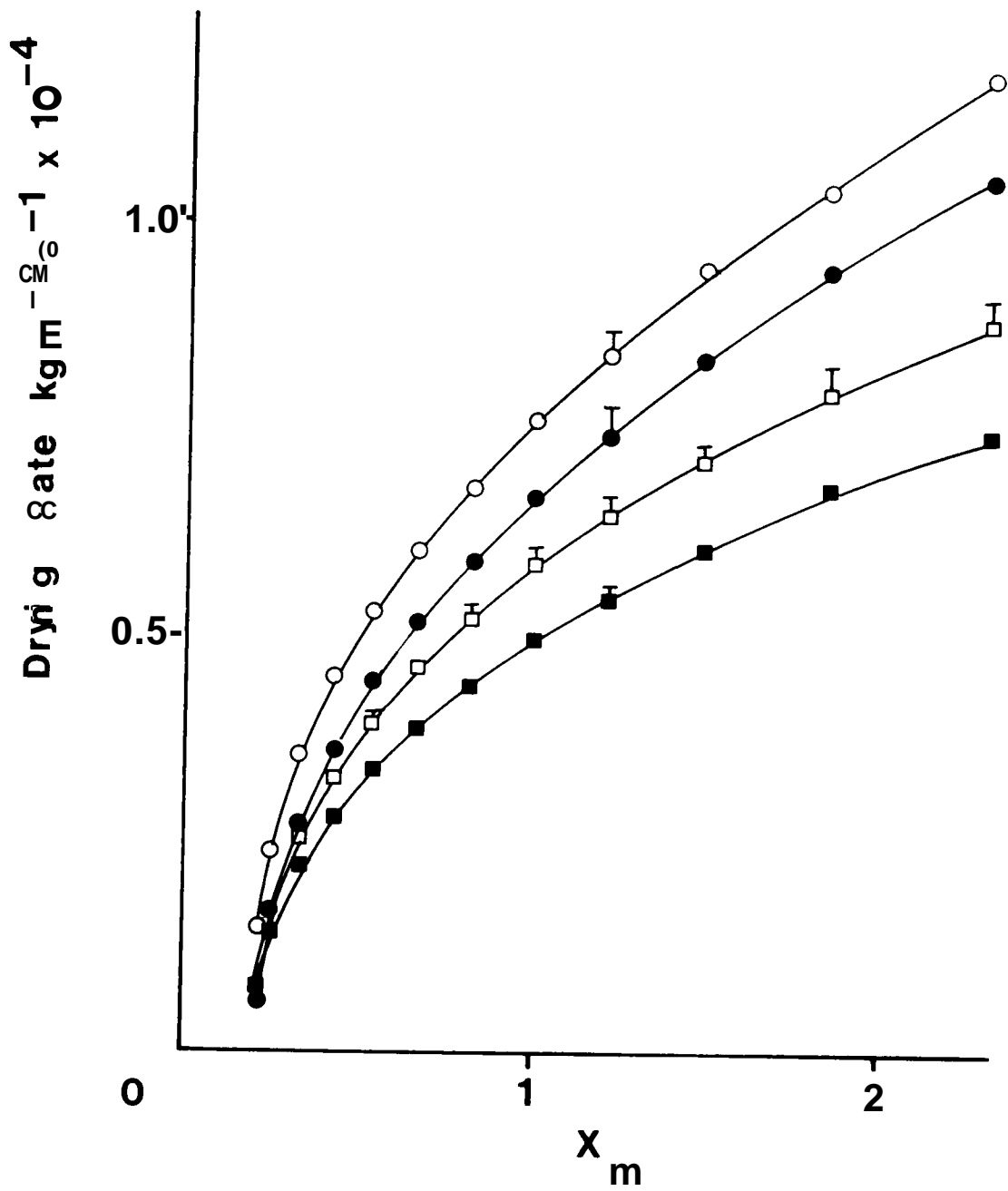


FIGURE 4.7

The Variation of Drying Rate with Humidity

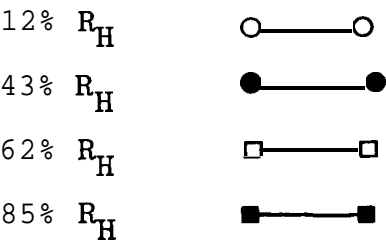
Gelatin Batch 7

Mean + 1SD (N=4)

Film Thickness : 0.400 - 0.410 mm

Temperature : 25°C

Humidity :



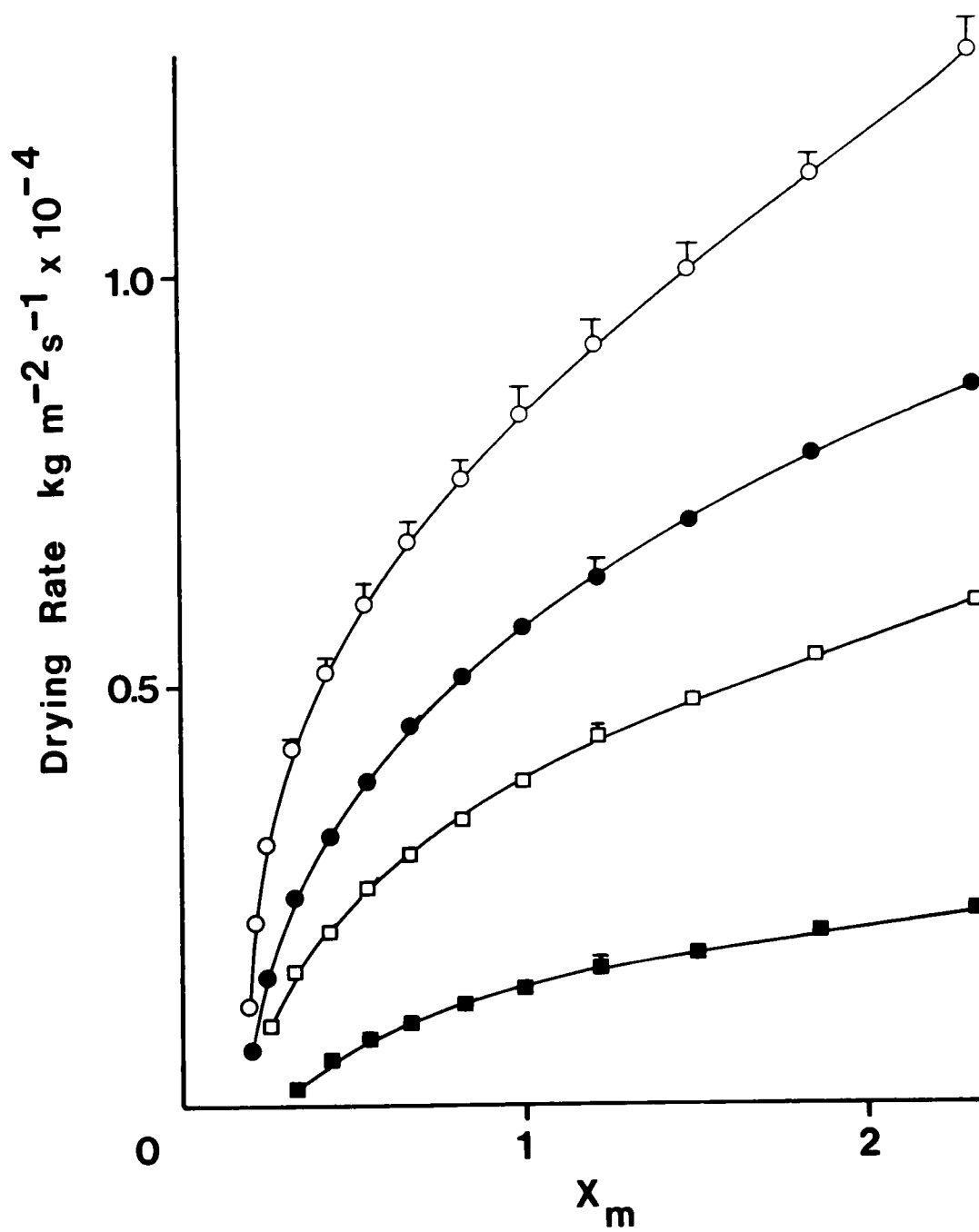


FIGURE 4.8

The Variation of Drying Rate with Gelatin Type

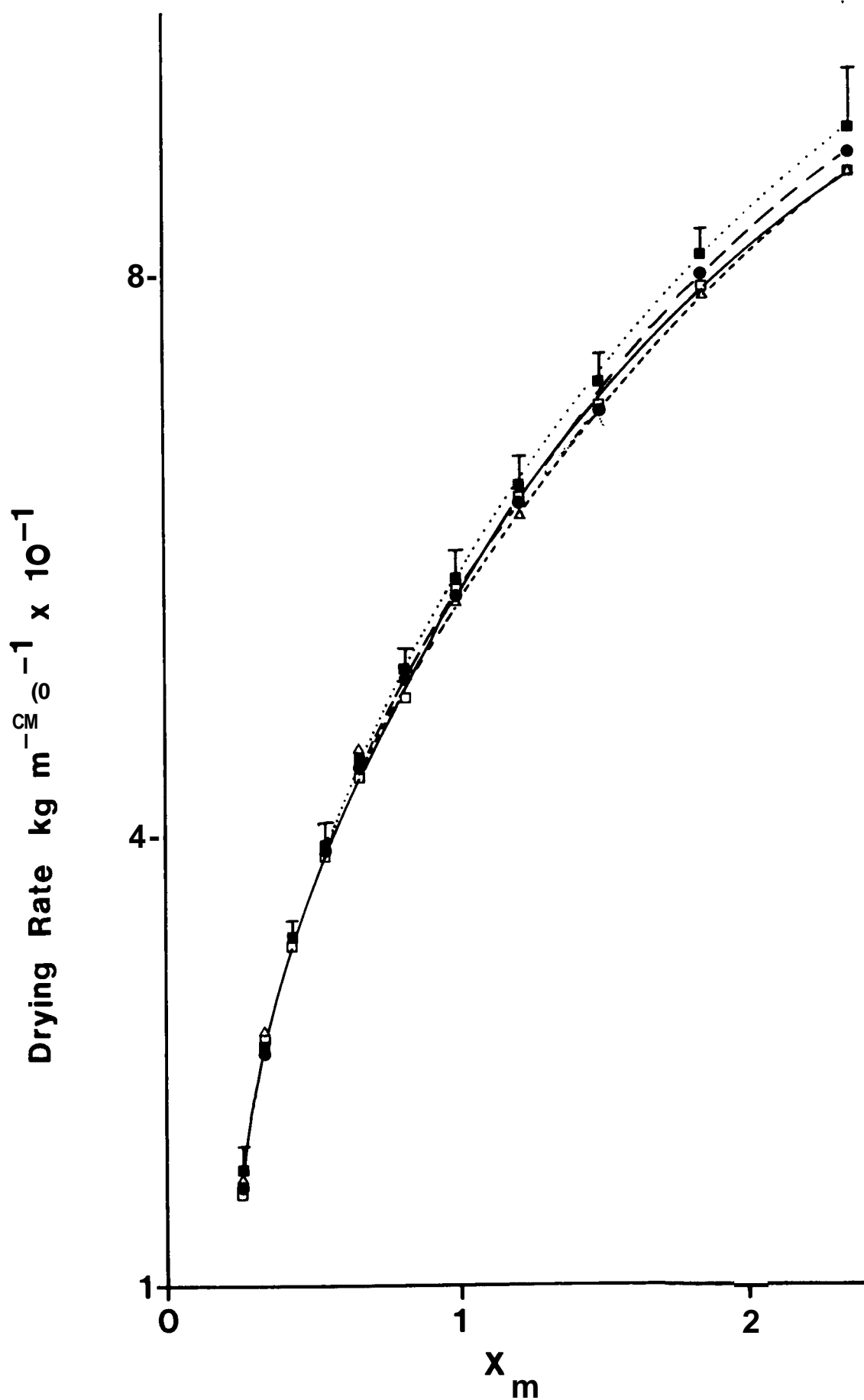
Mean + 1SD (N=4)

Thickness : 0.400 - 0.410 mm

Temperature : 25 °C

Humidity : 43% R_{rr}

'Good' Limed Ossein Gelatin (Batch 2)	□ — □
'Poor' Limed Ossein Gelatin (Batch 6)	● — — ●
Acid Ossein Gelatin (Batch 7)	△ — △
Acid Pigskin Gelatin (Batch 9)	■ •



Gelatin Type	Batch	Drying Time (min)	
		Mean	SD
'Good' Limed Ossein	2	68.3	1.60
'Poor' Limed Ossein	5	66.3	1.21
Acid Ossein	7	65.2	0.56
Acid Pigskin	8	64.9	1.52

Table 4.9 Mean Drying Times between Moisture Contents
of $X_m = 1.9$ to 0.25 in the Slow Drying Experiments

n = 4

structural development may be postulated. It was impracticable to drastically increase air velocities in the above apparatus because of balance instability and the use of lower humidities would alter the temperature of the drying sample.

An experiment was therefore designed to dry freshly-cast gelatin sheet under airflow conditions sufficient to achieve the industrial drying time.

4,3 FAST DRYING EXPERIMENTS

4.3.1 Apparatus

Open glass tube : length **1m**, internal diameter 75mm.

Aluminium plate : **square, flat** plate with rim 5mm
wide 0.5mm high. Overall dimensions
73 x 73 mm.

Hairdryers : Model NEHD11, EMEB, Nottingham, UK.

Anemometer : Thermo-anemometer GGA235
Wallace Oy, Turku, Finland.

Cyanoacrylate : Loctite (UK) Ltd.,
glue Welwyn Garden City
U.K.

4.3.2 Method

The experiment was carried out at $20 \pm 1^{\circ}\text{C}$ and $42 \pm 3\% R_H$ within the humidity and temperature controlled room (section 2.4). The tube was mounted horizontally and the two dryers arranged at one end to blow unheated air down the tube. Air velocity was measured at the opposite end by the anemometer probe placed just inside the tube. Air velocity was $3.9 \pm 0.1 \text{ m.s}^{-1}$ to within 5 mm of the tube wall.

The internal surface of the plate was roughened with emery paper and a thin ring of glue smeared around the rim. The plate was weighed, heated and placed on a levelled surface, A 25% w/w hot gelatin solution was poured onto the plate and the excess removed while still molten with the edge of a steel **rule**. Film thickness was controlled by weighing the plate 5 minutes after casting, A gel weight of 2,15 to 2,35 g gave a film thickness of 0.41 ± 0.02 mm. Under and **overweight, or** blemished films were **rejected**, 8 min after casting, the plate was inserted in the tube and driers switched on. At intervals of 4 min. the plate was removed, reweighed and replaced, rotating the plate 90° each time to avoid leading edge effects. Weighing took 20 s. On completion of drying a portion of film was cut from the centre of the plate, weighed and subjected to moisture determination.

4.3.3 Results and Discussion

Fig. 4.10 shows a typical drying curve under the experimental conditions. The apparent constant rate period is surprising and may result from a combination of cruder technique and faster drying rates making the initial curvature less easy to observe. However, although the moisture content is below the critical moisture content for mature gels, the immaturity of these freshly cast gels may aid moisture transport and the maintenance of a wet surface. In this case, the initial curvature seen in the slow-drying experiments may be explained as a result of age or shrinkage. A comparison of the slope of

FIGURE 4,10

The Drying of Gelatin Film in the Fast Drying Experiments

Gelatin Batch 7

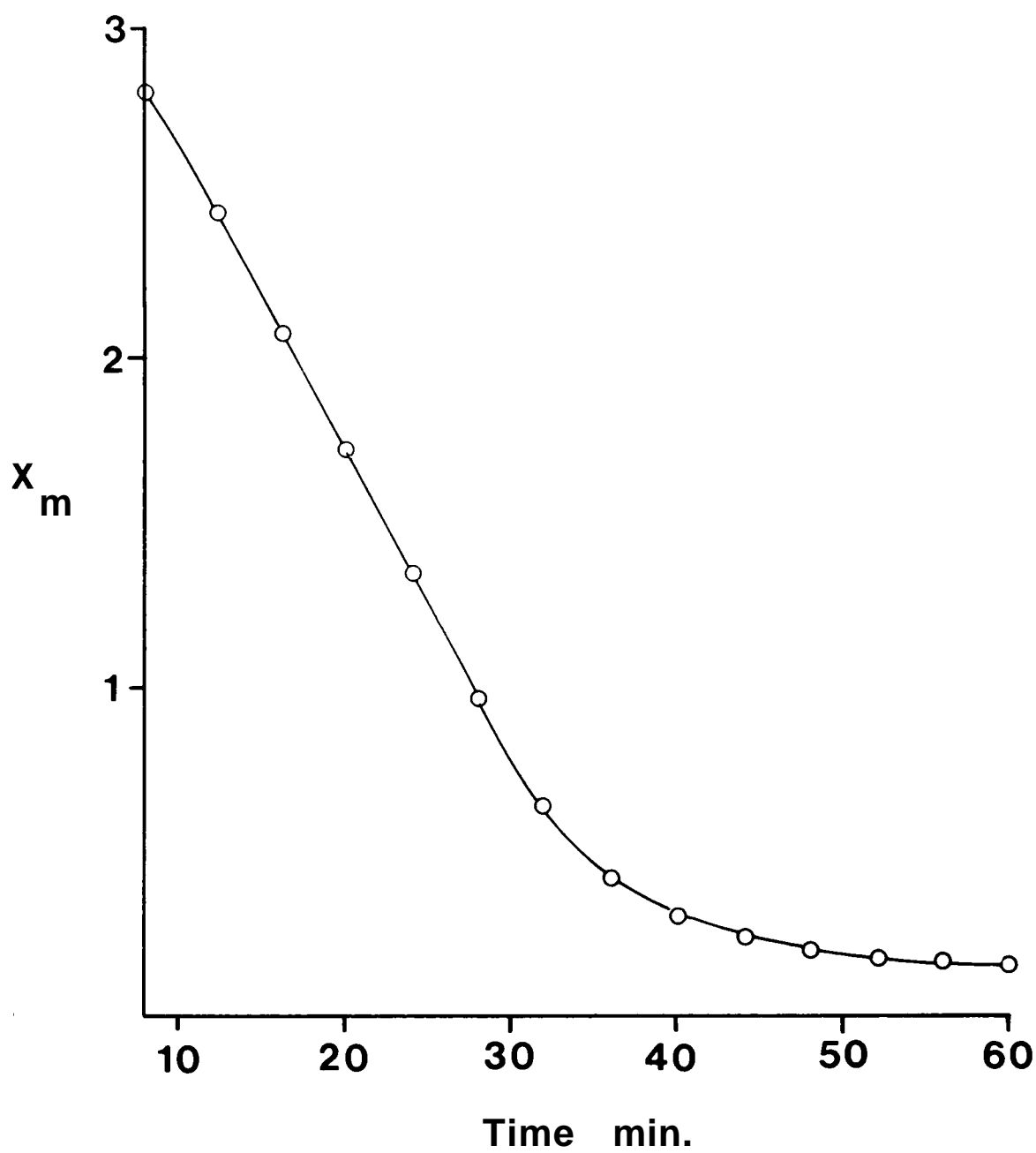
Temperature : 20°C

Humidity : $43 \pm 3\% \text{ R}_\text{H}$

Air velocity : 3.9 m.s^{-1}

Film Thickness : 0,42 mm

X_m : mean moisture content kg.kg^{-1}



Gelatin Type	Batch	Drying Time (min)	
		Mean	SD
'Good' Limed Ossein	2	43,8	2,2
'Poor' Limed Ossein	6	43,1	3.3
Acid Ossein	7	39.4	2.3
Acid Pigskin	9	40.7	2.9

Table 4.11 Drying times between $X_m = 2,3$ to 0.19
in the Fast drying experiments
n = 4

the linear portion ($1,8 \times 10^{-4} \text{ kg.s}^{-1}\text{m}^{-2}$) with the drying rate of a film of water ($3,0 \times 10^{-4} \text{ kg.s}^{-1}\text{m}^{-2}$) determined under the same conditions suggested that drying was not taking place from a wet surface.

The drying times between moisture contents corresponding to those of the capsule before and after drying ($X_m = 2.3$ to 0.19 : 70% to 16% wet weight basis) are compared in Table 4,11, The times at $X_m = 0,19$ were considerably dependent on the moisture content determinations which were therefore performed **together**. Paired t-tests at $p = 0,05$ revealed no significant differences between the four batches within experimental **error**. This result does not entirely preclude the possibility of varietal differences in drying rates because of the low precision of the **experiment**. The difference in mean drying time between the 'good' limed ossein and the acid ossein batch was 4.4 minutes. Compared with an overall drying time of 40 minutes a saving of 10% in drying time may well be regarded as industrially worthwhile.

CHAPTER V

THE EQUILIBRIUM MOISTURE CONTENT OF GELATIN FILMS

5.1 INTRODUCTION

The proportion of moisture that cannot be removed from a hygroscopic material by drying in humid air is termed the equilibrium moisture content (EMC) at that humidity (69). The EMC of gelatin is markedly dependent on humidity, and to a lesser degree upon pH, temperature, molecular weight, molecular structure and whether equilibrium has been achieved by sorption or desorption or by cycling between the two.

The sorption and desorption isotherms are sigmoidal in shape; typical of those for many proteins, polymers and gels (116-8, 135, 138) and may be analysed by the equations of adsorption. Below 45% R_H the data is well fitted by the B.E.T. equation (115,118), Above 45% R_H the equation of **Harkins** and Jura (119) is applicable. Values of the monolayer capacity (V_m) calculated using the two equations from different portions of the isotherm agree well (120). However, the use of adsorption equations implies a surface phenomenon whereas in **gelatin**, moisture sorption occurs throughout the entire mass. The quantity V_m has therefore been interpreted at the molecular level, being the quantity of water bound when each hydrophilic site is occupied by one water molecule (118).

Other authors (55, 121) have analysed sorption isotherms of gelatin by means of the **Flory-Huggins** equation for polymer solutions. Below 26% w/w moisture content the interaction parameter χ has a value greater than 0.5, indicating the favouring of polymer : polymer interactions and the co-existence of two separate phases (122). Only in the range 80 to 100% R_H may the sorption be regarded as solution ($\chi = 0.5$) (118).

From 0 to 100% R_H water molecules are bound in decreasing order of adsorption enthalpy (123). Nuclear magnetic resonance studies (116,122,124) indicate that below 10% R_H , water is very rigidly **bound, first** as single molecules and then as clusters. Completion of the Langmuir monolayer has been calculated to occur at about 20% R_H (125) with an increase in conductivity due to the net of water molecules covering the surface (116).

Above 80% R_H binding sites become saturated and further moisture is increasingly directed to **form** multilayers of condensed moisture (125). There is evidence for the appearance of new water binding sites as polymer : polymer interactions decrease (47, 122) and it has been shown that there is a failure of large numbers of such bonds at these high humidity levels.

The main sites for the binding of water on proteins are (115) :

- (i) The oxygen and nitrogen atoms of the peptide backbone chains and side groups.

- (ii) Side-chain polar groups of e.g. lysine, tyrosine and glutamic acid.

Of these, (ii) when ionized have a disproportionate effect on EMC due to their high binding capacity (6-8 water molecules) relative to (i) (one water molecule each) (126-129). The EMC of gelatin increases with pH, particularly from pH2 to 6, and a consideration of the groups involved led Pouradier (118) to conclude that the increase in EMC could be almost entirely explained by the increasing ionization of carboxyl groups on the molecule. At pH6 ionized carboxyl groups carry over 20% of the total monolayer capacity of gelatin, though they are present only to the extent of 1.2 m.moles per gram.

Studies of degraded gelatins (130) and gelatin fractions (118) indicate that molecular weight has little influence on the EMC. This is predictable as only at very low molecular weights would the binding capacity of increased numbers of end groups become significant relative to the total capacity of the polymer side groups.

The degree of structural order within a film does, however, appear to influence the EMC. At 60% R_H , which is the point of maximum separation of the isotherms, collagen (almost 100% triple-helix) has an EMC approximately 8% higher, and cold-dried gelatin film (partially renatured) approximately 2% higher, than hot-dried gelatin film (random coil) (29,55). This has been attributed to the triple-helical

structure orientating backbone peptide groups in a configuration more favourable to water binding (131).

Little work has been done on varietal differences in EMC. Sheppard et al (130) have noted a lower EMC for an acid pigskin gelatin compared with a limed hide gelatin above 60% R_H .

Hysteresis, the separation of sorption and desorption isotherms on cycling between low and high R_H has often been reported and Rao and co-workers have indicated varietal differences between gelatin batches. (132-138) Unfortunately no details of tissue of origin or **manufacturing** process are **given**. Hysteresis in gelatins has been interpreted using the theory of Young and Nelson (139) by York (125) in terms of the balance of binding and diffusional forces at the sorting surface, or by the cavity concept of Rao (135). There is evidence of a decrease in total sorptive capacity in some gelatins on repeated cycling (125,138) and this effect has been attributed to a shift in the pore-size distribution (123).

The final stage of drying of hard gelatin capsules takes place after manufacture (2). The following experiments are designed to examine gelatin films for batch differences in EMC. If these do occur, can they be related to differences in gelatin type and known suitability for hard capsule manufacture?

5.2 EXPERIMENTAL

5.2.1 Apparatus

- (A) Individual desiccators) as described in section 2.4.1
- (B) Saturated salt solutions)
- (C) Oven-Incubator hybrid with internal fan) as described
) in section
- (D) Thermocouples for temperature monitoring) 4.2
- (E) Analytical balance reading to four decimal places.

A.Sauter:Model 414/8

5.2.2 Method

Desiccators were maintained for 2 days under experimental conditions prior to use. Dried gelatin films were prepared (as section 2.2) of thickness 0.14 to 0.18 mm and pieces were cut 25 x 120 mm . They were placed one in each desiccator and were conditioned at $T \pm 0.2$ C. Temperature uniformity was monitored by means of the thermocouples placed in different parts of the oven and gelatins to be compared were conditioned at the same time. Desiccators were then taken from the oven and the strips weighed. Moisture **determinations** were then performed under conditions, and with the precautions for randomizing error due to atmospheric exposure, described in 2.3.

Ten replicates were performed for each experimental point.

5.3 RESULTS AND DISCUSSIONS

5.3.1 The Variation of Film Moisture Content with Conditioning Time

The attainment of equilibrium of films conditioned

at 33% R_H and 20°C is shown in Figure 5.1. The moisture content values are statistically indistinguishable after 3 days. Likewise there is no significant difference between moisture contents at 7 and 14 days at 12% R_H and 76% R_H (Table 5.2). At 86% R_H , the highest humidity, attainment of equilibrium could not be verified as the moisture contents of films conditioned for longer than 7 days were considered unreliable because of microbiological **spoilage**. Conditioning time was standardised at 7 days in subsequent experiments,

5.3.2 The Variation of EMC with humidity

Figure 5,3 shows the effect of humidity on the EMC of films of a batch representative of each gelatin type. Samples conditioned at the same R_H were subjected to moisture determinations together and are thereby comparable. At all R_H values the EMC of the acid pigskin batch is **significantly** lower (T-test, $p = 0.05$) than the 'good' limed ossein batch. The other gelatins lie between the two.

The rank order changes at 86% R_H but attainment of equilibrium could not be verified at this humidity.

Further experiments were performed at 33% R_H , this being within the range optimum for the storage and use of hard capsules (46,47).

5.3.3 The Variation of EMC with Temperature

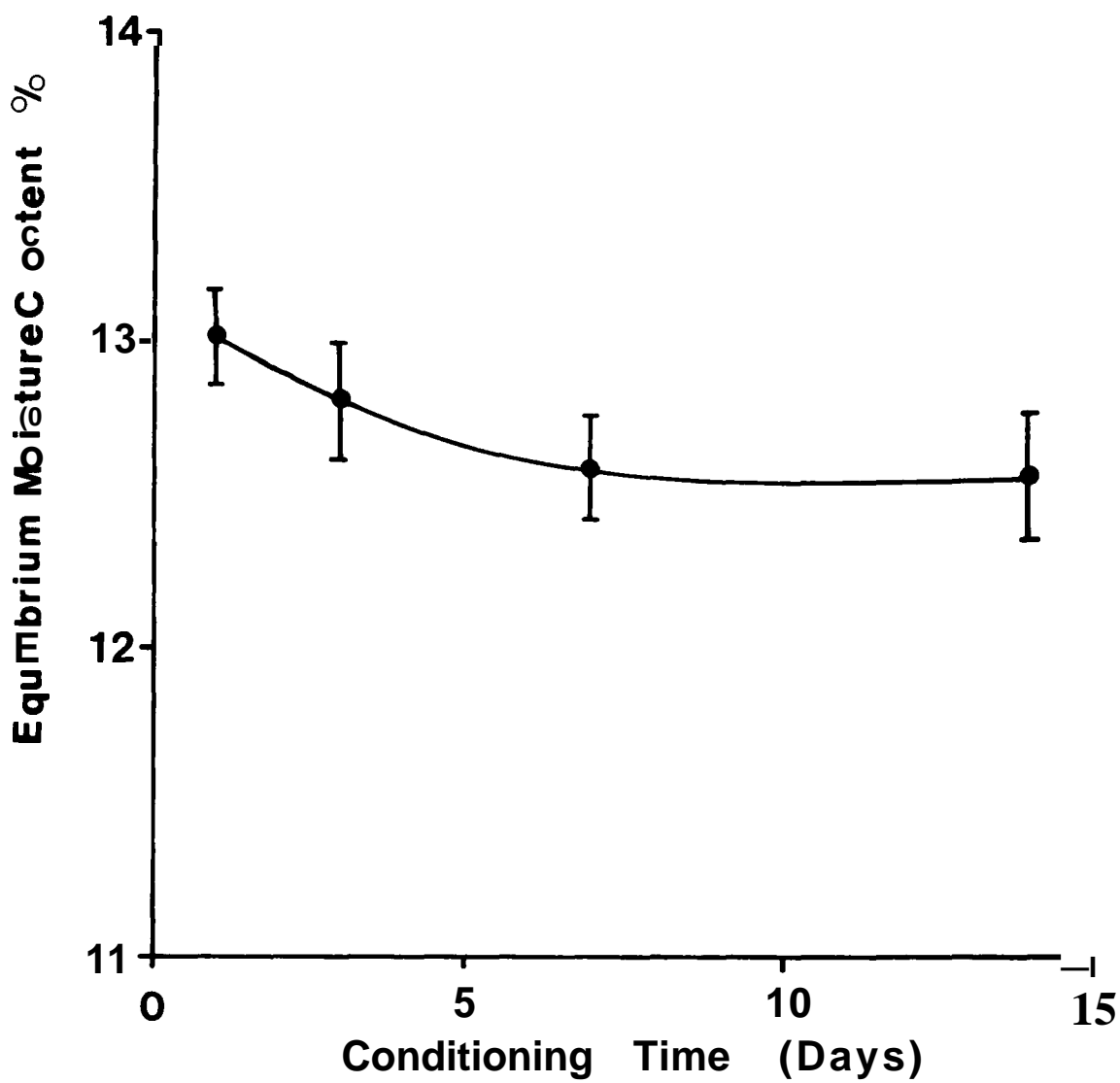
Figure 5.4 shows that the EMC of all the gelatin films decreased with temperature by approximately 1.5%

FIGURE 5.1

The Variation of Moisture Content of Gelatin Film
With Conditioning Time at 33% R_H and 20°C.

Gelatin Batch 7

Mean ± 1 **std.dev.** (N = 10)



HUMIDITY % $R_{T,H}$	MOISTURE CONTENT %			
	7 DAYS		14 DAYS	
	MEAN	STD.DEV.	MEAN	STD.DEV.
12	7.77	0.21	7.70	0.13
76	19.73	0.14	19.68	0.14
86	23.14	0.15	Spoilage	

TABLE 5.2 The Moisture Contents of Films Conditioned
for 7 and 14 days; at High and Low Humidities at
20°C.

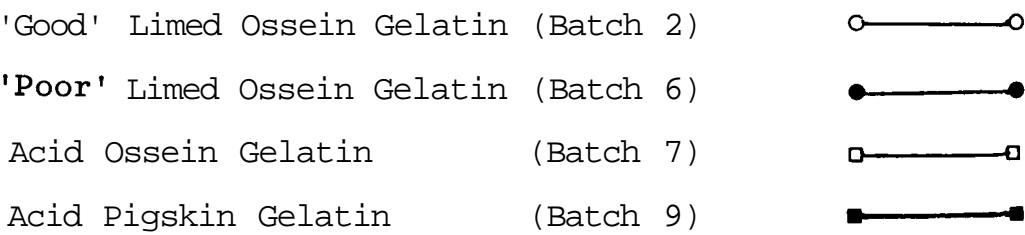
Gelatin Batch 7

N = 10

FIGURE 5.3

The Variation of Equilibrium moisture content with
Relative Humidity at 20°C.

Mean ± 1 std.dev.(N=10)



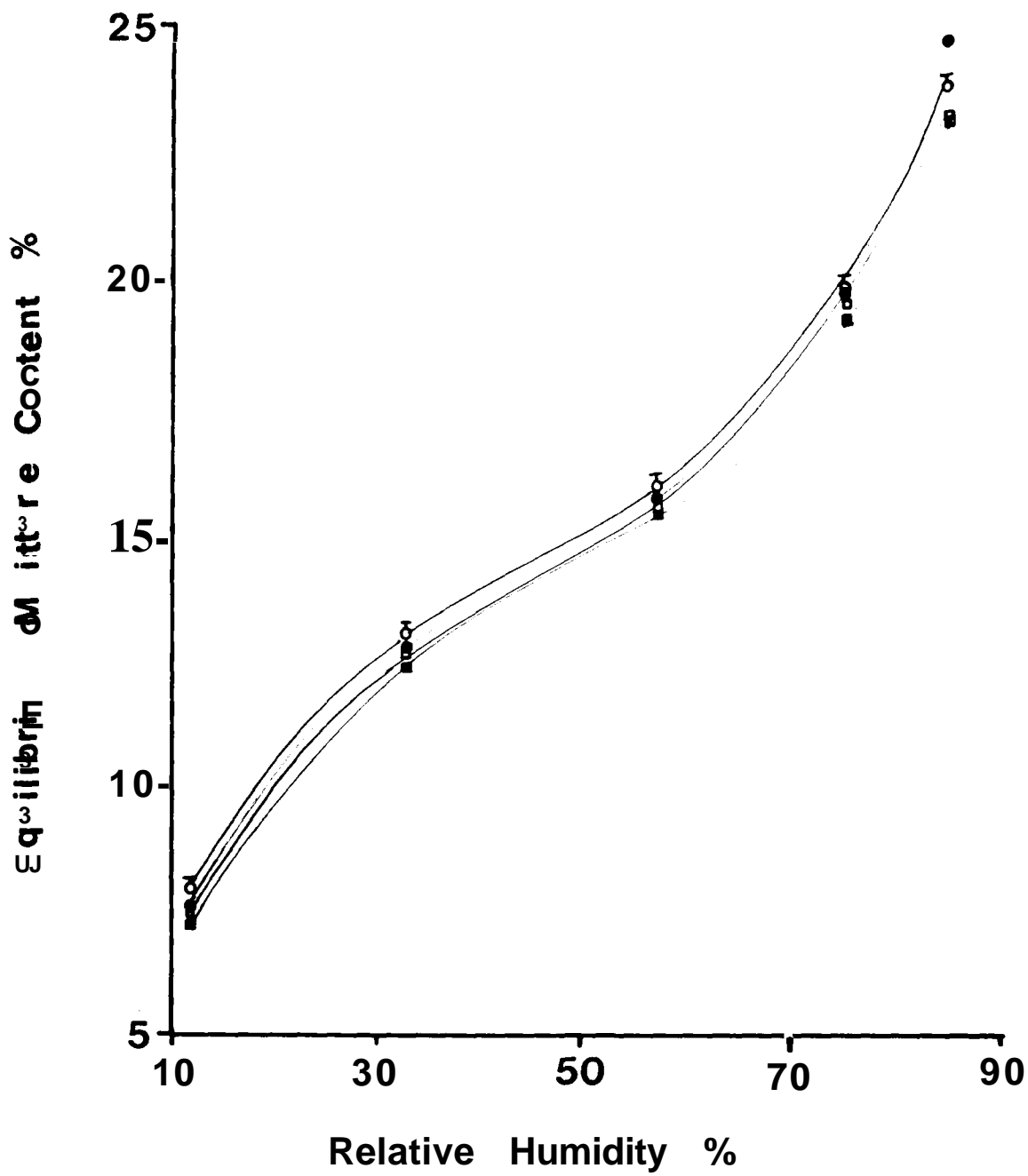
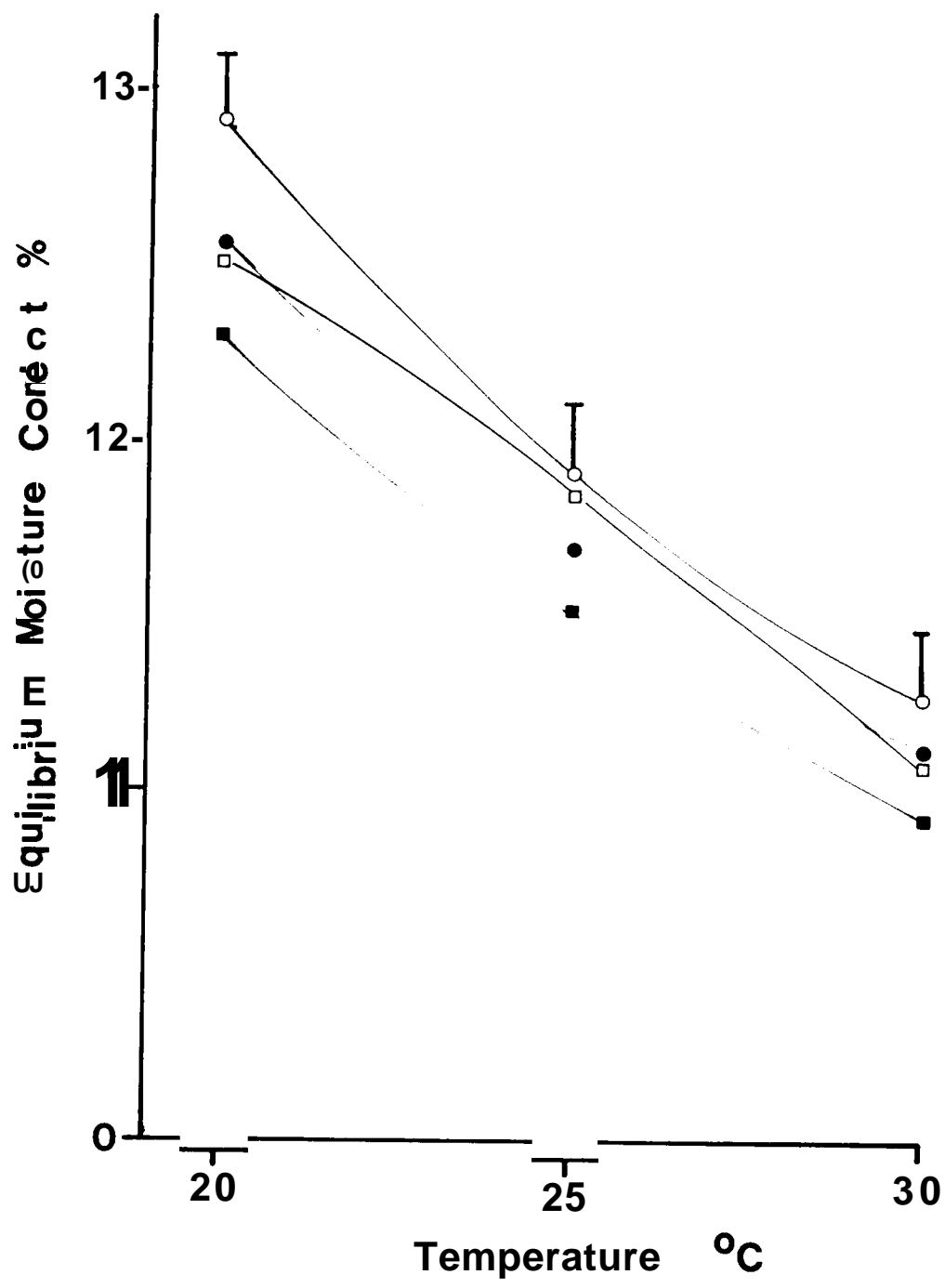


FIGURE 5.4

The Variation of Equilibrium Moisture Content with
Temperature at 33% R_H

Mean \pm 1 std.dev. (N=10)

'Good' Limed Ossein Gelatin (Batch 2)	○——○
'Poor' Limed Ossein Gelatin (Batch 6)	●——●
Acid Ossein Gelatin (Batch 7)	□——□
Acid Pigskin Gelatin (Batch 9)	■——■



over the range 20 to 30°C. This is a consequence of the thermodynamic equilibrium existing between gelatin-bound water molecules and water vapour. Ghermann and Kast (140) have noted a lowering of the EMC over the whole sorption isotherm between 20, 40 and 60°C although superimposed on these results is the effect of the triple helix-coil reversion which occurs at around 40°C.

The rank order of gelatins is similar to that of 5.2.2.

5.3.4 The Variation of EMC with Gelatin Batch

The EMC values at 33% R_{rr} and 20°C of the full range of gelatin batches were determined in an attempt to elucidate whether the differences already observed were due merely to batch variation or could be more generally ascribed to gelatin type.

The largest difference in EMC (Table 5.5(A)) was of the order of 0.6% and statistical testing (Scheffe Range Test, $p = 0.05$) showed the only significant difference to be between batches 2 and 9.

The ash contents of these gelatins are of the same order (0.1 to 0.7%) as the difference in EMC. If the results are recalculated adjusting for ash content the spread of EMC values becomes greater. The differences in EMC are therefore not attributable solely to differing ash contents (Table 5.5(B)).

Much of the error associated with these results can be attributed to slight variations in the conditions during moisture determination runs. For each run the different gelatins emerged in similar rank order but their mean EMC was shifted higher or lower with respect to other runs. This source of variation may be removed by subtraction of the run mean from each result. Table 5.5(C) shows the effect of this transformation in reducing the error with respect to the differences between gelatins.

The non-homogeneity of variances (max/min variance ratio = 8.3. $p < 0.01$) demands a non-parametric test for the comparison of these values. Employing a Mann-Whitney U test at $p = 0.01$, groups of gelatins significantly different from each other are shown in Table 5.6. The groups divide the gelatin batches neatly into their respective types, suggesting that gelatin type may be influencing the EMC.

The possibility exists that these small but significant differences in EMC are related to pH through the proportion of carboxyl groups that are ionised. Dilute solution pH values, which provide a good indication of film pH (118) show no correlation with values of EMC (Correlation coefficient $r = 0.3$) and pH cannot account for the difference between batches 5 and 9 which have pH values of 5.79 and 5.80 respectively. Likewise, ash contents may affect EMC. Although no significant direct correlation exists ($r = 0.61$, $p > 0.05$), considering the error associated with the determination of each, this possibility must be borne in mind.

Type	Batch	Equilibrium Moisture Content (%)					
		(A)		(B)		(C)	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Limed ossein (good)	2	12.91	0.18	12.99	0.18	+0.35	0.08
	3	12.60	0.21	12.67	0.21	+0.03	0.07
Limed ossein (poor)	4	12.69	0.17	12.77	0.17	+0.13	0.05
	5	12.61	0.24	12.71	0.24	+0.07	0.08
	6	12.56	0.22	12.67	0.22	+0.02	0.07
Acid ossein	7	12.51	0.26	12.52	0.26	-0.12	0.15
	8	12.49	0.18	12.50	0.18	-0.14	0.08
Acid pigskin	9	12.29	0.18	12.31	0.18	-0.33	0.11

Table 5.5 The Variation of Equilibrium Moisture Content with Gelatin Batch

n = 10. Films conditioned at 33% R and 20^o C.
H

(A) Data as obtained

(B) Data adjusted for ash content

(C) Data adjusted as (B) and calculated as a difference from the mean EMC for each moisture determination run.

GROUP	BATCH	GELATIN TYPE
1	2	'Good' Limed Ossein
2	3	'Good' Limed Ossein
	4	'Poor' Limed Ossein
	5	'Poor' Limed Ossein
	6	'Poor' Limed Ossein
3	7	Acid Ossein
	8	Acid Ossein
4	9	Acid pigskin

TABLE 5. 6 Gelatin Batches divided into **Significantly**
Different Groups on a Basis of EMC Values
by the Mann-Whitney U-test at $p = 0.01$

Due to pretreatment during manufacture, acid and lime processed gelatins differ in their carboxyl group contents (Section 1,2) and when ionized, these groups are major binding sites for **water**. The rank order of decreasing carboxyl group content (as reflected by isoionic point) is the same as that for EMC namely limed osseins > Acid osseins > Acid **pigskin**. The difference in carboxyl content between an acid pigskin ($pI = 9,1$) and a limed gelatin ($pI = 4.92$) has been calculated as $0,38 \text{ m.moles.g}^{-1}$ (15) of which approximately 95% would be ionised at pH 5,8 (142), The difference in EMC determined here, between an acid pigskin (batch 9) and a typical limed gelatin (batch 3) is $0,31 \text{ m.moles.g}^{-1}$; well within the binding capacity of the extra carboxyl **groups available**. The differences in EMC may therefore be explained in terms of differing carboxyl group content, except for batch 2 which has an idiosyncratically high EMC. It is possible that the low EMC of the pigskin gelatin examined could also be merely a batch variation and the above conclusions can only be tentative.

The 'poor' limed osseins were not **significantly** different from batch 3, a proven 'good' limed **ossein**, and their EMC gives no indication as to the nature of their inability to form good capsules.

5.3.5 The Effect of Blending on EMC

The effect of changing the proportions of a blend of an acid and a limed ossein gelatin on EMC is shown in

Figure 5.7. The EMC appears lower at the higher concentrations of acid ossein than would be expected if each gelatin contributed proportionately to the EMC.

It would be imprudent to draw definite conclusions from these results as the measurements are at the limits of experimental precision and an analysis as 5.3.4 did not produce further **clarification**.

In a study of mixtures of food biopolymers, Iglasias et al (141) have found that where the EMC could not be predicted from proportional contribution it was invariably lower. This was attributed to the involvement of water binding sites in polymer: polymer interactions.

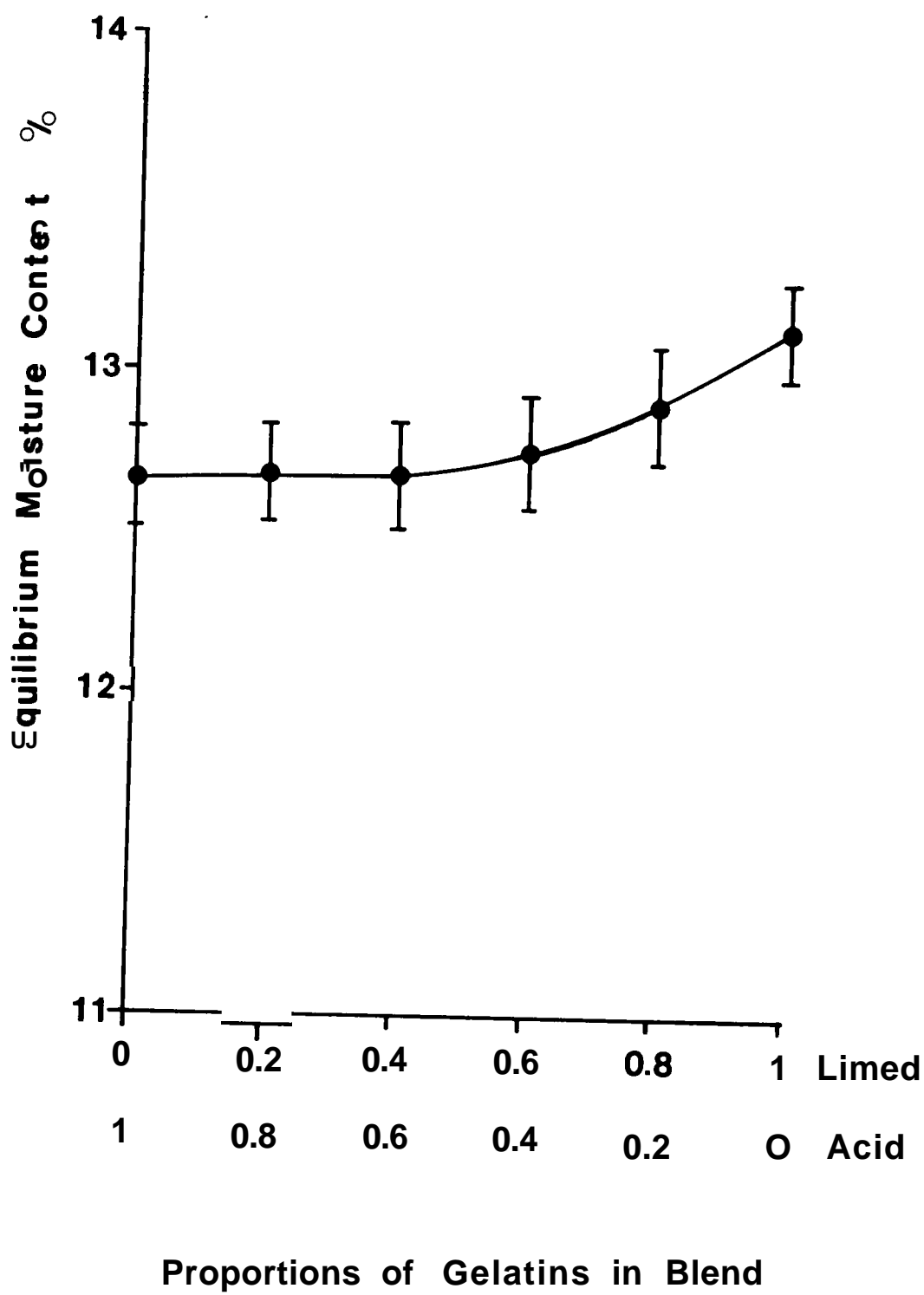
FIGURE 5.7

The Equilibrium Moisture Content at 33% R_H and 20°C
of Films of Acid and Limed Ossein gelatin Blended in
Different Proportions

Values adjusted for ash content

Mean \pm 1 std.dev. (N=10)

Gelatins:	Limed Ossein	Batch 2
	Acid Ossein	Batch 7



CHAPTER VI

CHAPTER VI

THE MECHANICAL TESTING OF GELATIN FILMS

6.1 INTRODUCTION

6.1.1 The Behaviour of Gelatin as a Viscoelastic Polymer in the Glass Transition Region

Yannas (169) has concluded that amorphous gelatin exhibits a major glass transition between 175 to 200°C and that therefore the viscoelastic behaviour of gelatin parallels that of synthetic polymers (170).

The glass transition temperature (T_g) is an important property of a polymer which marks the onset of free segmental movement of polymer chains and a sharp increase of the "free volume" within the material. At temperatures below T_g polymer chains are locked in fixed positions within a disordered lattice and molecular motion is restricted to vibrational modes. The polymer has high modulus (rigidity) but is brittle with low impact strength. This is termed the glassy state. At temperatures above T_g rubbery behaviour is exhibited, with low modulus and large recoverable extensions **possible**. Many applications (including hard **carsules**) require the material to possess a moderately high modulus combined with a lack of brittleness. Polymers exhibit these properties at temperatures around T_g (the transition region) and they may be chemically **modified**,

crystallized, blended or plasticized such that this region is within the working temperature of the material (170).

Plasticizers are low molecular mass substances that facilitate molecular movement and thereby reduce T_g (173), Water is a powerful plasticizer of gelatin films (it depresses T_g by up to 5°C per 1% (29)) and at temperatures from $0 - 120^{\circ}\text{C}$ the moisture content dominates the mechanical behaviour of gelatin (174),

An increase in plasticizer concentration alters gelatin mechanical properties in a manner equivalent to a rise in temperature (172,176), **devitrification** occurring at approximately 13% moisture at room temperature (175), The high intrinsic molecular flexibility conferred by the glycine molecules in the chains is manifest at high plasticizer concentrations ($> 27\%$ water (181)) when gelatin behaves as a highly elastic rubbery gel (197), An increase in free volume (55) and molecular freedom (124) with elevation of moisture content has been demonstrated for the gelatin-water system.

Polymers may be classified as amorphous (having a random molecular **configuration**) or semi-crystalline (containing areas of structural order). The two may be distinguished by X-ray **diffractometry**. Crystalline areas act as crosslinks, increasing the modulus in the transition region and extending over a greater range of temperature and plasticizer concentration, the useful region where high modulus and low brittleness coexist (170).

The presence of areas of renatured helical structure give "cold dried" gelatin film (CDF) the properties of a semicrystalline polymer containing areas of both amorphous and crystalline structure (177). In contrast, "hot dried" film (HDF) exhibits the diffuse X-ray pattern typical of amorphous polymers (22, 178) and contains very little structural order. CDF possesses a greater tensile strength and is less brittle than HDF at room temperature.

At 75% R_H and above, CDF has a much higher modulus but exhibits a lower elongation to break than HDF due to the restrictions on molecular movement imposed by the crystallite "crosslinks". (21,22,54). Further evidence of this crosslinking effect is the limited swelling of CDF in cold water (179). Crystallite regions may be induced in gelatin films by stretching in humidities $> 73\% R_H$ when the molecular orientation is sufficient for areas of triple helix to spontaneously form, (32, 54),

6,1.2 The Tensile testing of Polymer Films

The viscoelastic properties of polymers may be assessed by a multitude of testing procedures (180), the most important being impact, creep, stress-strain and stress relaxation experiments which may be performed in a tensile, compressive, shear or flexural mode (181). When the manufacturer assesses the flexibility of a capsule shell by squeezing the open end of a capsule half, he is performing a crude form of flexural stress-strain test: some areas of the shell are in

compression but, due to microscopic flaws in the gelatin, failure is more likely in areas under tension and a tensile test is more likely to give a representative indication of the strength in use. (180).

Uniaxial tensile stress-strain tests are widely used to evaluate polymer films (153) and this work will utilize this type of test along with some stress-relaxation studies.

A uniaxial stress-strain test involves the continuous measurement of uniaxial load as the specimen is deformed at a constant rate of elongation. The nominal stress is calculated from the load by normalization with respect to original cross-sectional area,

$$\sigma = \frac{F}{A_0}$$

σ : nominal stress
 F : uniaxial load
 A_0 : original cross-sectional area

and strain by dividing the elongation by the original length.

$$\epsilon = \frac{L - L_0}{L_0}$$

ϵ : strain
 L : specimen length
 L_0 : original specimen length

The tensile stress-strain modulus (Young's modulus), a measurement of rigidity, is the stress-strain ratio over the initial linear portion of the stress-strain curve for which Hooke's law is a good approximation.

$$E = \frac{\Delta \sigma}{\Delta \epsilon}$$

E : Tensile modulus

The tensile strength (ultimate breaking strength) of a material is the stress at fracture, whilst fracture toughness (a lack of brittleness) may be assessed from the

energy absorbed by the material before fracture, i.e. the area under the stress-strain curve.

The shape of the stress-strain curve provides information. Fig. 6.1 (a) shows the changes in shape of curve around the transition **region**. (i) is the glassy state whilst (iv) is typical rubbery behaviour of a polymer above T_g . (ii) and (iii) represent forms of behaviour in the transition region where moderately high modulus and toughness coexist. Although the tensile strength is lower than in the glassy state, greater elongations are possible because the material can dissipate by deformation the stress-concentrations around flaws that might otherwise exceed the breaking stress. The yield peaks that emerge mark the onset of a 'neck' over a portion of the specimen.

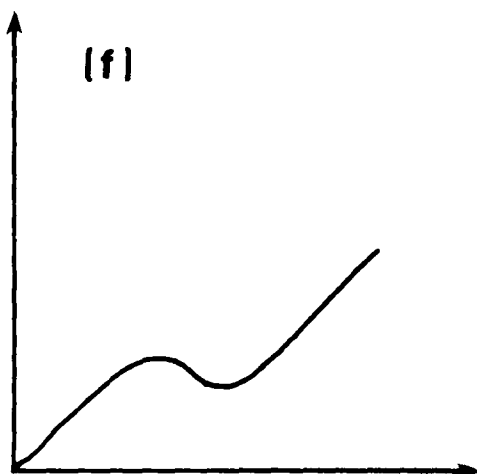
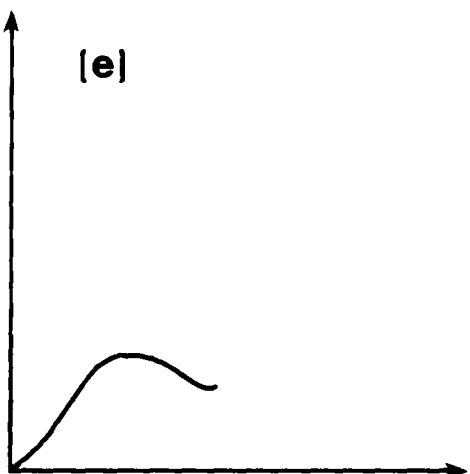
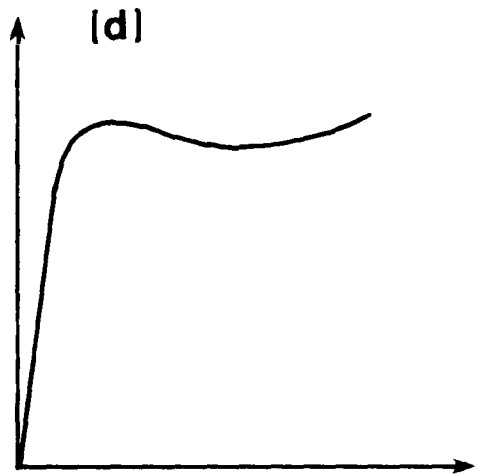
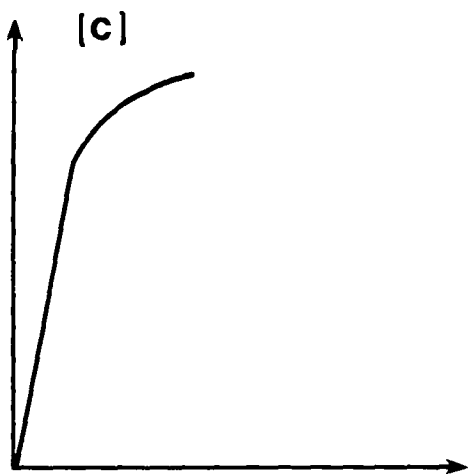
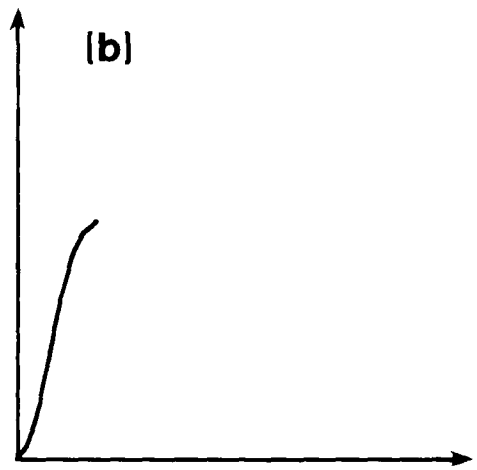
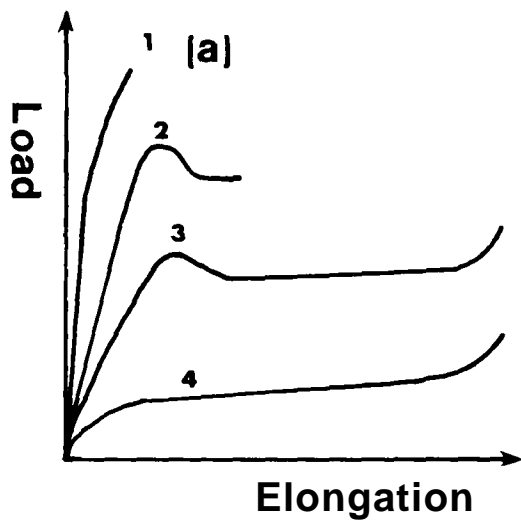
Other shapes of curves are evident for polymer behaviour and are shown in Figure 6.1 (b-f) classified according to their gross physical characteristics. The type of curve obtained is dependent upon the internal structure of the material, the degree of **plasticization**, the conditions and the timescale of testing.

Tensile stress-relaxation testing involves the rapid application of a small finite uniaxial strain and the measurement of the decay of the induced stress. It is a measure of the time-dependent behaviour of a material under load. The relaxation mechanisms involved may be analysed in terms of viscoelastic models (144). In this work which is purely

FIGURE 6.1

Types of Stress-Strain Curve (173,182,183)

- a) The Variation of Behaviour around T_g :
- | | |
|--------------------------------------|---------------------|
| 1, Brittle fracture | Glassy region |
| 2, Ductile behaviour |) |
| |) Transition region |
| 3, Cold-drawing after neck formation |) |
| 4, Elastomeric behaviour | Rubbery region |
- b) Hard and brittle - High modulus and moderately high fracture stress. Low elongation. No yield peak.
- c) Hard and strong - High modulus and fracture stress. Moderate elongation. No yield peak.
- d) Hard and tough - High modulus, fracture stress and elongation. Yield peak.
- e) Soft and weak - Low modulus and fracture stress. Moderate elongation. Yield peak.
- f) Soft and tough - Low modulus. Moderate fracture stress. Large elongation. Yield peak.



comparative, the stress relaxation modulus measured 60 seconds after application of the strain is used (144). For a material exhibiting linear viscoelastic behaviour this is defined as

$$E_r(60) = \frac{\sigma(60)}{\epsilon}$$

$E_r(60)$: 60-second stress relaxation modulus
 $\sigma(60)$: stress at 60 seconds
 ϵ : applied strain

6.2 APPARATUS

- (A) Instron Bench Model Tensile Tester,
- (B) Self-Tightening Elastomeric jaws, 2713-001

Both the above obtained from:

Instron Ltd,, High Wycombe, U.K,

- (C) Micrometer Model 961M, Moore & Wright, Sheffield, UK,

A rig was built for mounting the micrometer such that film widths could be measured.

- (D) Individual desiccators)
 - (E) Saturated salt solutions)
- } as described in
Section 2.4,1,

6.3 STRESS STRAIN EXPERIMENTS

6.3.1 Method

Dry gelatin films of thickness 0.13 - 0.18 mm, were prepared as described in section 2.2. Particular care was taken to prevent film flaws caused by air bubbles, inadequate solution mixing (streaky films) and airborne dust particles.

Test strips of film were cut of width 5 mm and length approximately 110 mm. The guillotine produced a better edge and a more uniform sample width than either a hand-held scalpel or a die-stamping machine. Care was taken to minimise surface scratching during cutting and fingermarks were avoided by handling the strips with plastic gloves. Specimens were visually inspected against a bright background and those exhibiting marks, notches, streaks or other abberations were rejected.

The strips were conditioned at $18 \pm 2^{\circ}\text{C}$ over moist salts or phosphorous pentoxide in individual desiccators, maintained as in section 2,4,1. Prior to testing, sample width and thickness was measured at three points along the gauge length using a micrometer. The sample was rejected if variation of more than 3% in thickness or 1.5% in width was apparent. Two marks 94 mm, apart were made outside the gauge length before mounting in the centre of the jaws. Aligning these marks with the edge of the roller-mounting bar provided a gauge length of 50 mm.

A stress-strain test to fracture was carried out and the load-elongation curve recorded on the instrument. Samples that slipped or failed at the grips or around a prominent stress-concentrating abberation were rejected. Ten strips were tested for each experimental point. For those experiments conducted at the standardised strain rate of $1 \times 10^{-2} \text{ s}^{-1}$, exposure to room conditions up to the end of the test was not more than 3 minutes.

Moisture determinations were performed in triplicate on 250 x 120 mm. samples cut from the same films and conditioned concurrently. To allow for moisture loss or gain to the atmosphere during testing, moisture determination samples were exposed to room conditions for an equivalent time to the strips before weighing.

6.3.2 Results and Discussion

6.3.2.1 The Load-Elongation Curve

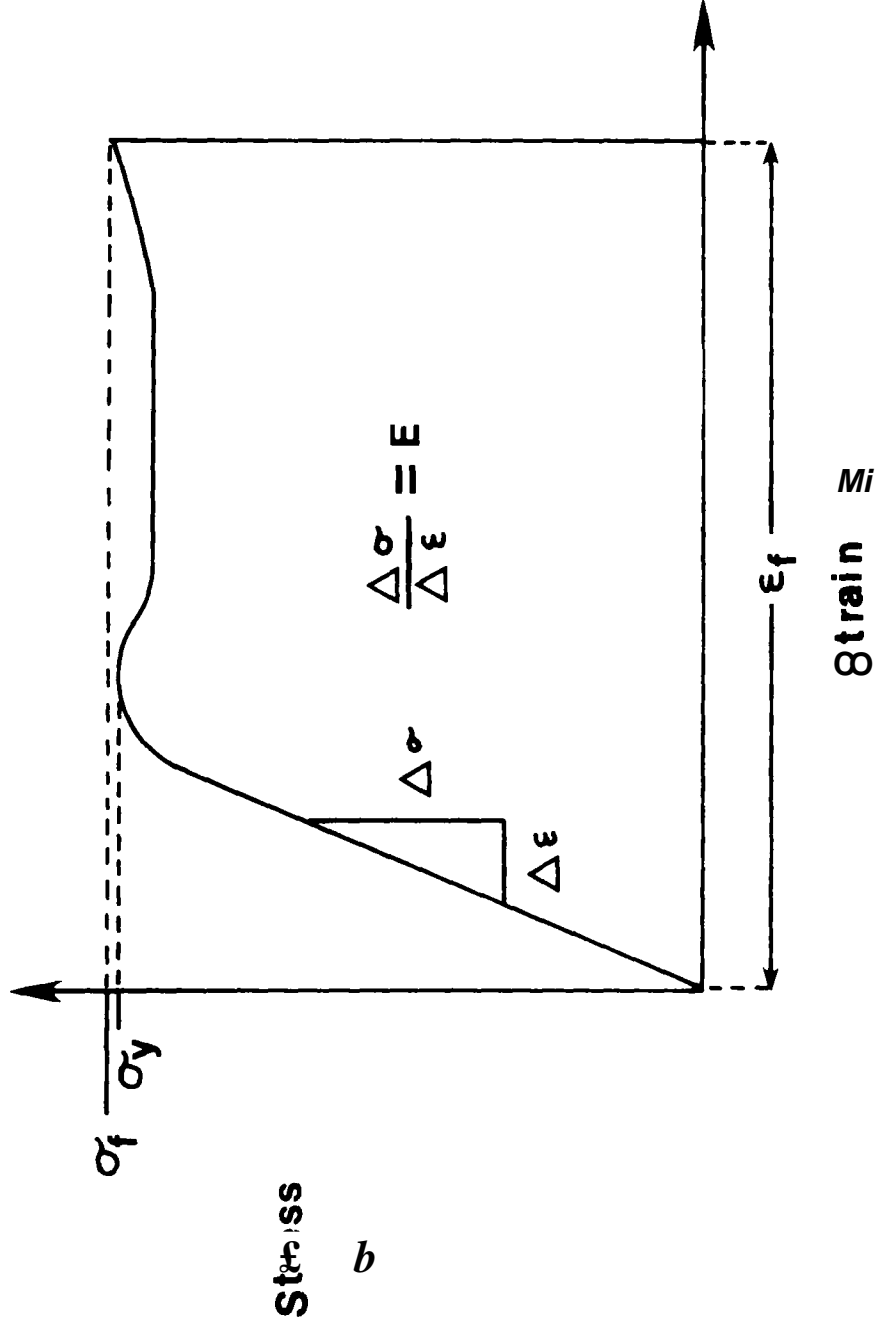
An idealised stress-strain curve, is shown in Figure 6.2. Four parameters were used to characterize the curve : the tensile modulus (TM), the stress at the yield peak (YSTS), the fracture stress (FSTS) and the fracture strain (FSTN). The curve shape varied with moisture content and some examples are shown in Figure 6.7 and discussed in 6.3.2.4. An inherent problem is the inaccuracies in the measurement of strain at low values from tightening of the grips and grip-holders (181). The delicacy of these films prevented the cementing of strain gauges onto the sample. These experiments were comparative and the assumption was made that this source of systematic **error** and others were the same for all experiments. However, strain values are more correctly described as 'nominal strain'.

Prior to a comparison of gelatins, experiments were conducted to establish a suitable strain rate for testing and sample ageing time.

FIGURE 6.2

An Idealised Stress-Strain Curve (182)

σ_f	:	fracture stress
σ_y	:	stress at yield peak
ϵ_f	:	fracture strain
E	:	tensile modulus



6.3.2.2 The Effect of Strain Rate

The effect of strain rate on TM and FSTS for films conditioned at the extremes of humidity (0 and 88% R_H) are shown in Figures 6.3 and 6.4. These parameters varied little over the range of strain rates investigated (0.3 to $1.7 \times 10^{-2} \text{ s}^{-1}$), which is **unusual**, as for most viscoelastic polymers, these parameters usually increase with strain rate (153). Similar values for the TM of gelatin films have been reported at lower strain rates (56). The strain rate was standardized at $1 \times 10^{-2} \text{ s}^{-1}$ in subsequent experiments,

6.3.2.3 The Effect of Ageing

Strips were conditioned for 1, 3 and 7 days prior to testing. Ageing has little effect on TM and FSTS (Figures 6.5 and 6.6) although an increase in TM is seen after 3 days at the highest moisture content (24%). It may be postulated that this may be due to continued helical growth augmenting crystallite areas at a plasticizer concentration that allows sufficient freedom of molecular movement (191). Ageing time was standardized at 3 days in subsequent experiments.

6.3.2.4 The Effect of Moisture Content

Films of a 'good' limed ossein, an acid ossein and an acid pigskin batch were tested over a wide range of moisture contents.

FIGURE 6.3

The Variation of Tensile Modulus with Strain Rate

Gelatin Batch 7

Mean \pm 1 SD (N=7 to 10)

Conditioning Time : 3 days

Conditioning Humidity :

0% R_H ○ ——— ○

88% R_H ● ——— ●

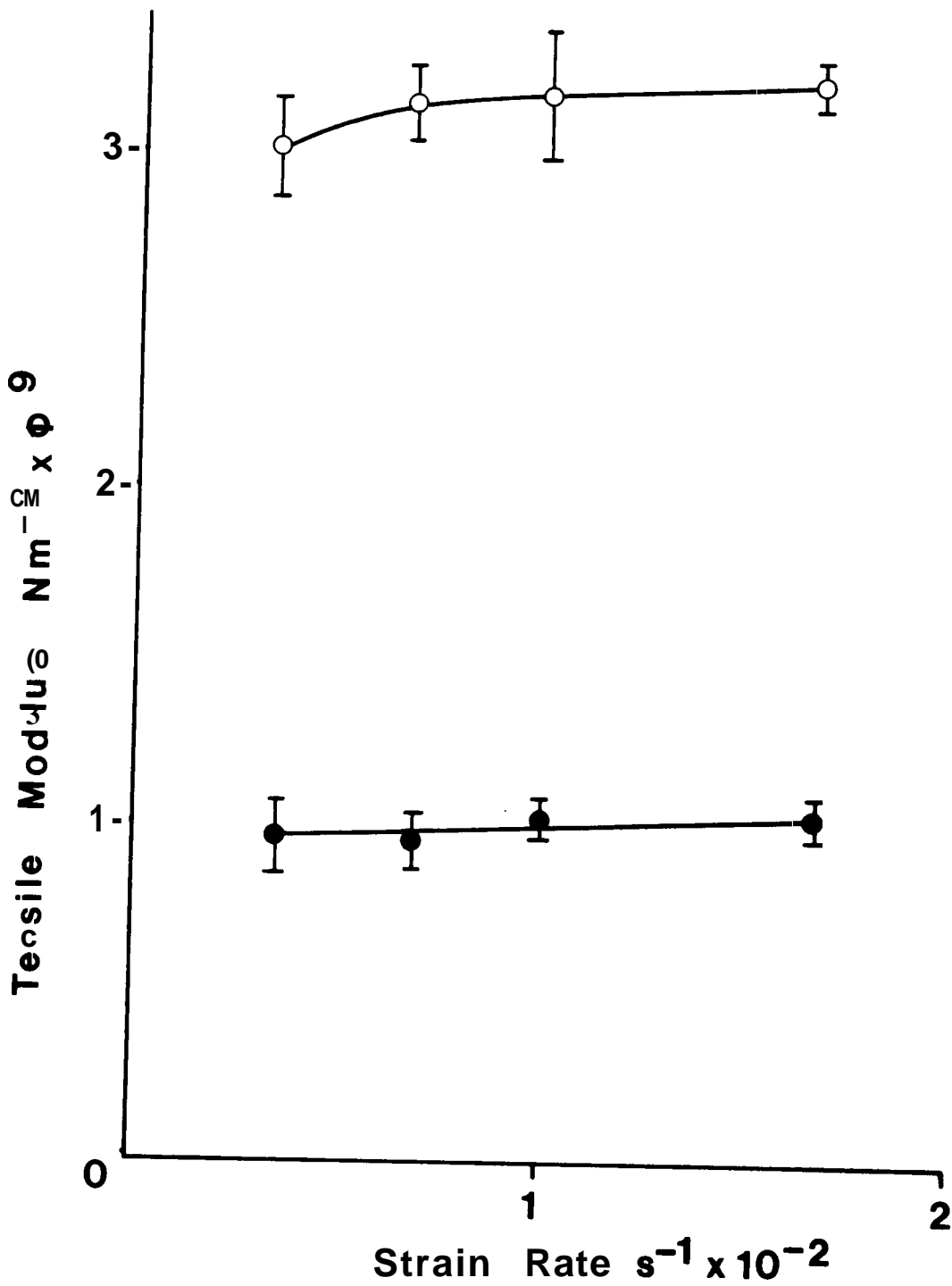


FIGURE 6.4

The Variation of Fracture Stress with Strain Rate

Gelatin Batch 7

Mean \pm 1 SD (N=8 to 10)

Conditioning Time : 3 days

Conditioning Humidity :

0% RH ○ — ○

88% RH ● — ●

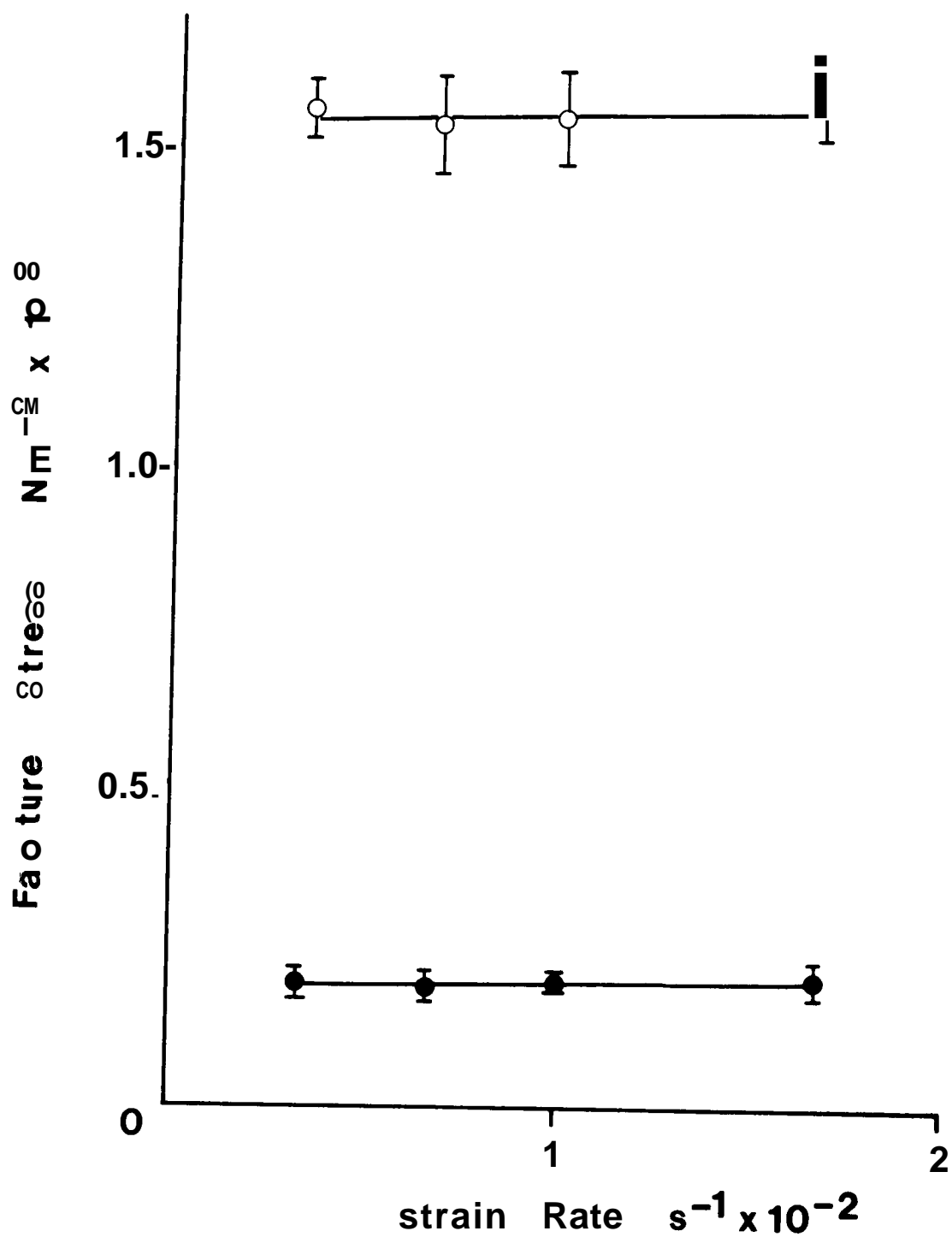


FIGURE 6.5

The Variation of Tensile Modulus with Sample Ageing Time

Gelatin Batch 7

Mean + 1 SD (N=8 to 10)

Conditioning humidity : 33%

Conditioning time:

1 Day ● — ●

3 Days □ □

5 Days ▲ —▲

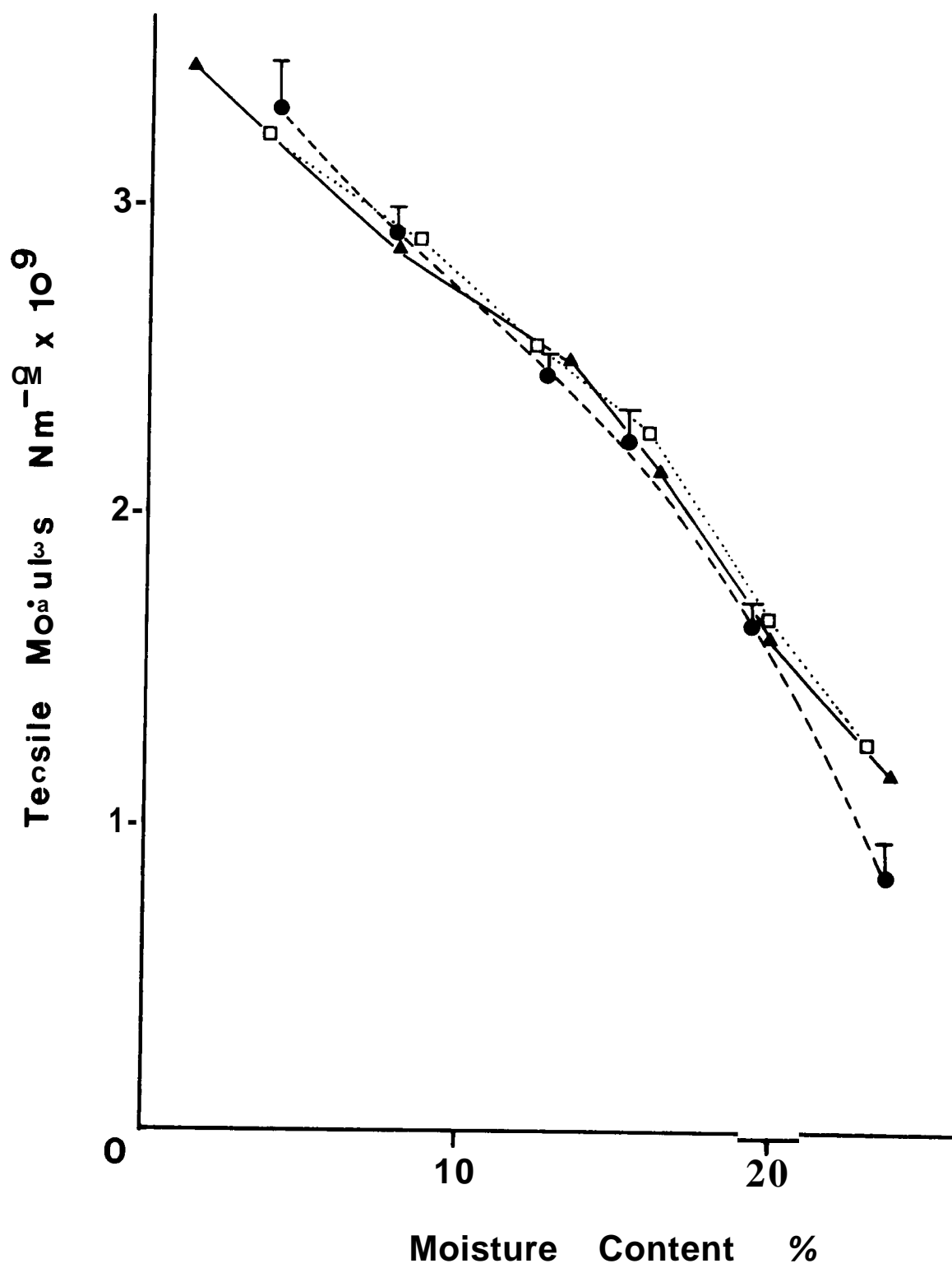
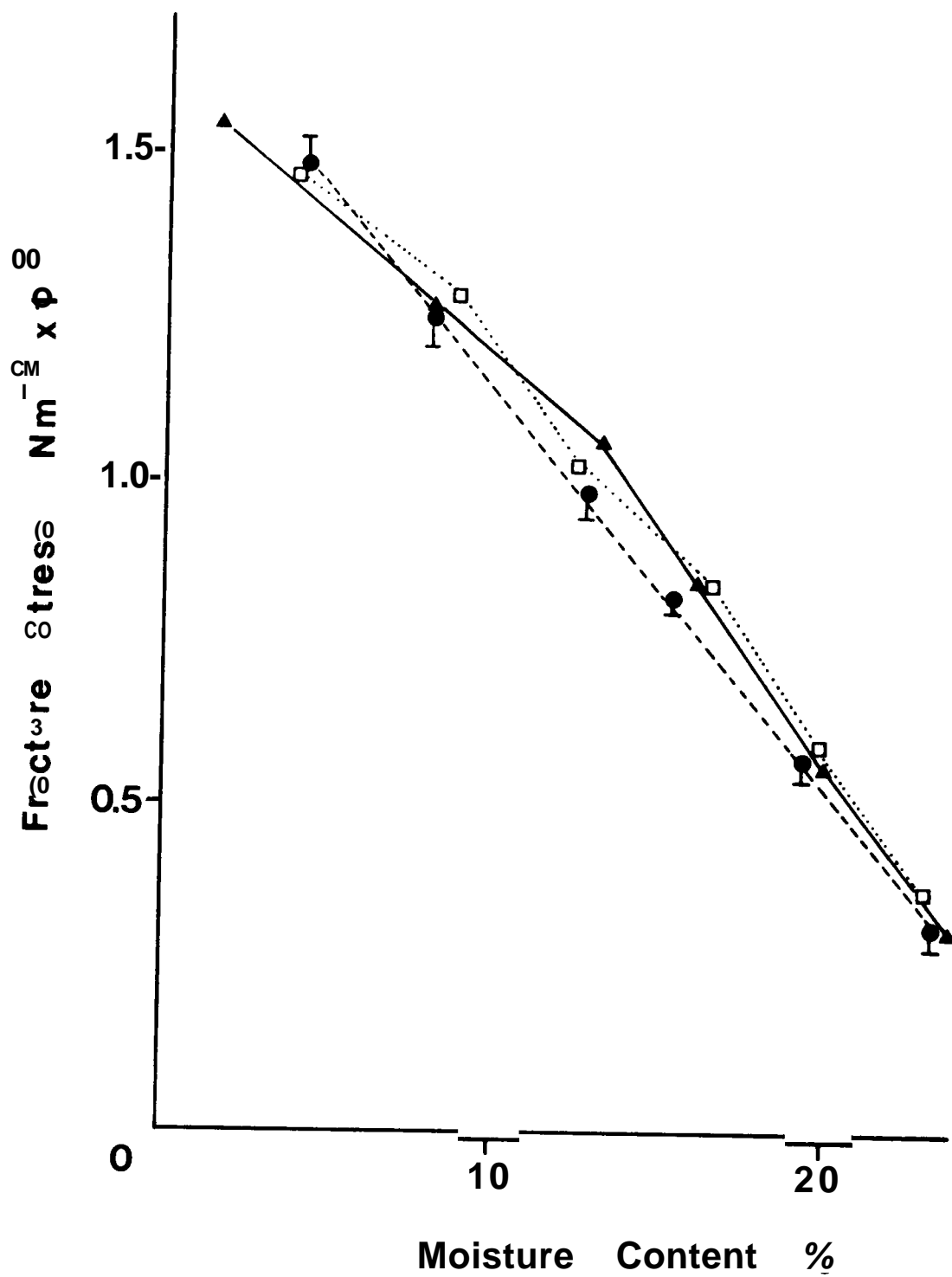


FIGURE 6.6

The Variation of Fracture Stress'with Sample Ageing Time

Gelatin Batch 7
Mean + 1 SD (N=8 to 10)
Conditioning Humidity : 33% R_{rr}
Conditioning Time : n

- 1 Day ●————●
- 3 Days □.....•
- 5 Days ▲————▲



The shape of the load-elongation curve was dependent on moisture content. At 3% and 8% moisture, curves were of the "hard-strong" type with moderate elongation to break and no yield peak. (Fig. 6.7(i)). At 13% moisture a yield peak emerged and a large elongation to break was observed (Fig. 6.7 (2)). These traces were of the "hard-tough" type. At 24% moisture content the film exhibited only a small initial linear portion before plastic deformation and there was a low extension to break. At this level of plasticization the films are comparatively soft and weak.

TM decreased from approximately $3.5 \times 10^9 \text{ Nm}^{-2}$ at 3% to $1 \times 10^9 \text{ Nm}^{-2}$ at 24% moisture content (Fig. 6,8), These values agree well with other workers (34, 184) who used lower strain rates, but the maximum reported to occur between 8 - 15% moisture was not observed in these experiments (34, 56), No differences were apparent between the different gelatin types,

FSTS decreased from 1.8×10^8 to $4.0 \times 10^7 \text{ Nm}^2$ and at 3% and 8% moisture content the limed ossein possessed a higher FSTS than the acid ossein batch. (Fig.6.9). This difference was small but significant (U-test : $p < 0.01$).

Yield peaks were observed at moisture contents of 13% and above, and YSTS values followed closely those of FSTS with no significant differences between gelatins (Fig, 6,10),

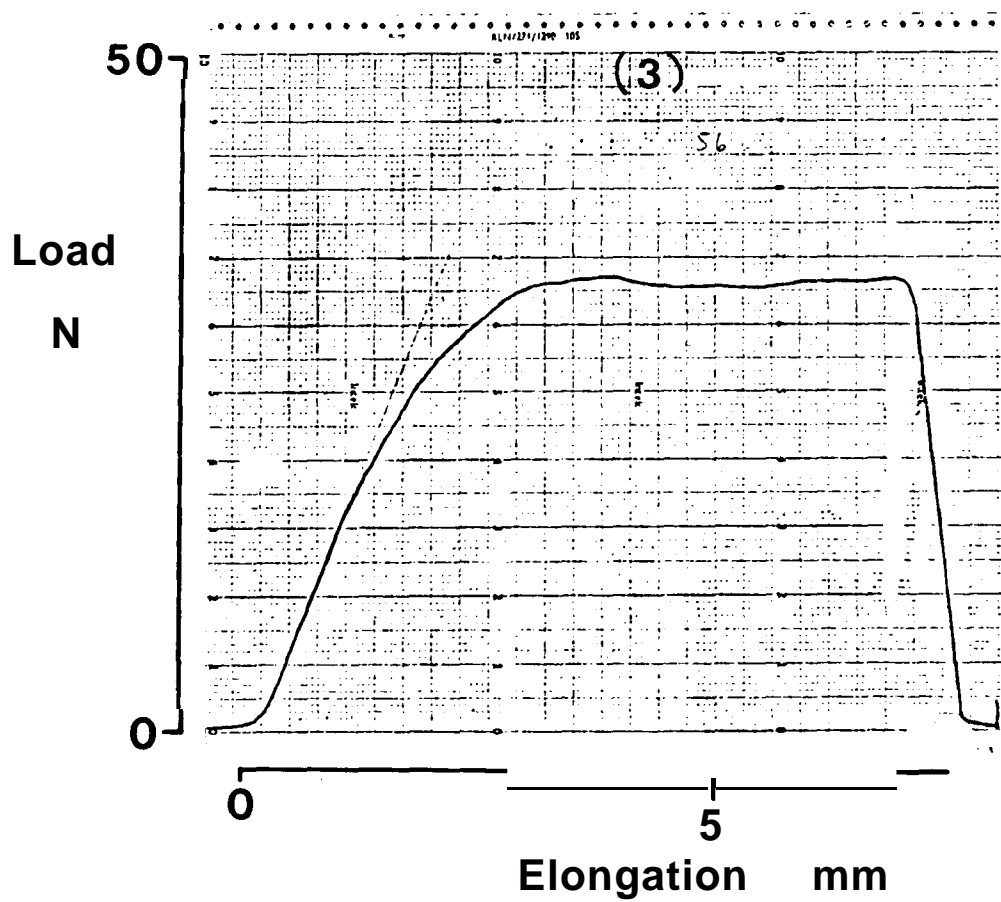
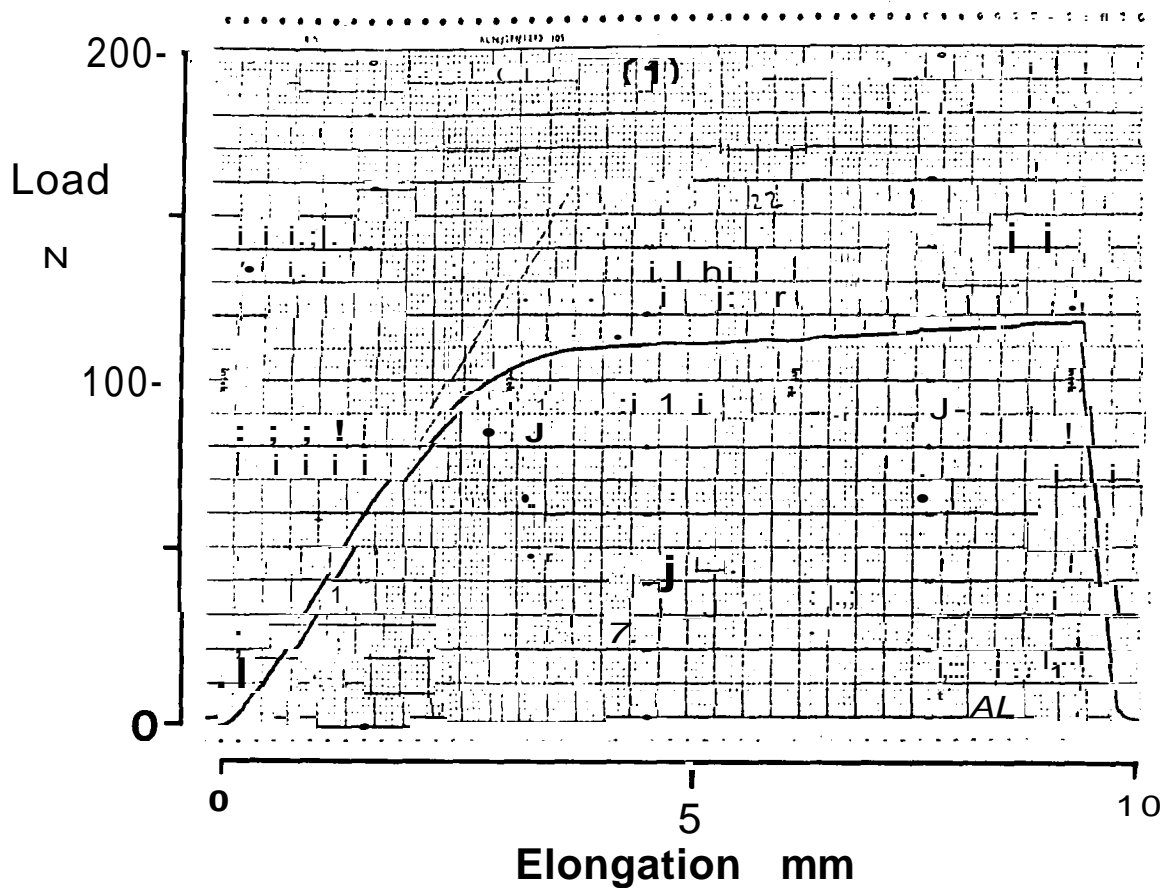
FIGURE 6.7

The Variation of Load-Elongation Curve with Moisture Content

Gelatin Batch 2. Strain rate $1 \times 10^{-2} \text{ m s}^{-1}$.
3 days conditioning.

- (1) 3% moisture content
- (2) 13% moisture content (overleaf)
- (3) 24% moisture content

Mean Cross-sectional areas: (1) 0.675 mm
(2) 0.711 mm
(3) 0.939 mm



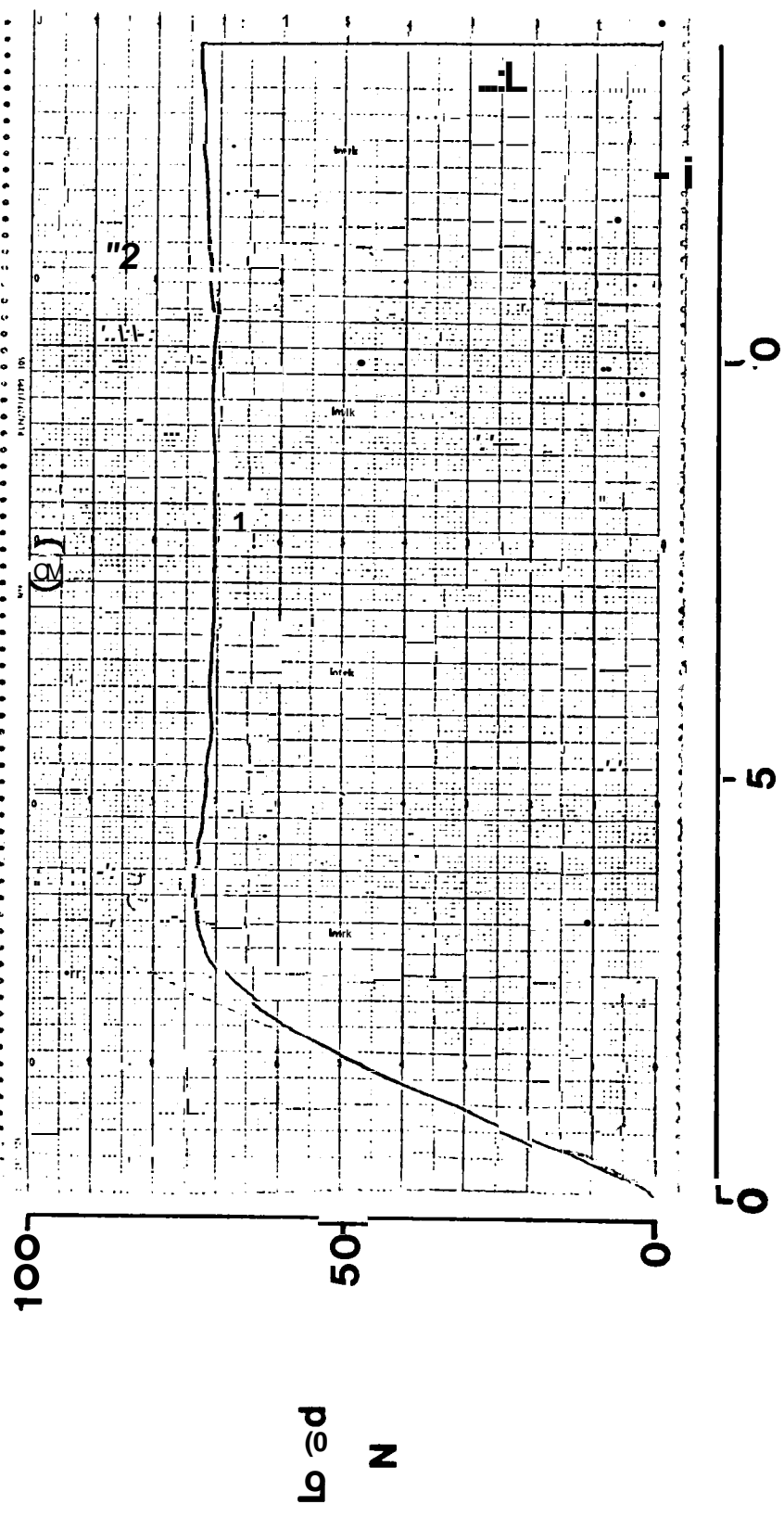
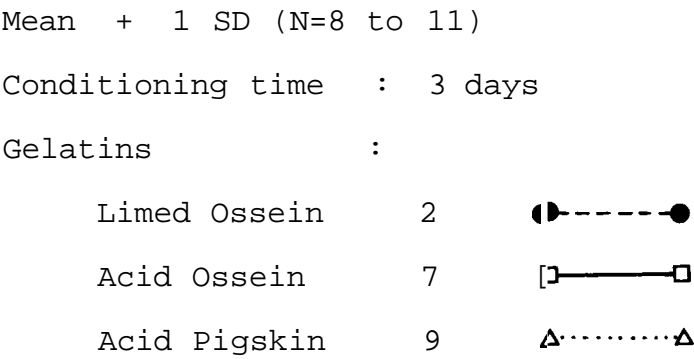


FIGURE 6.8

The Variation of Tensile Modulus with Moisture Content



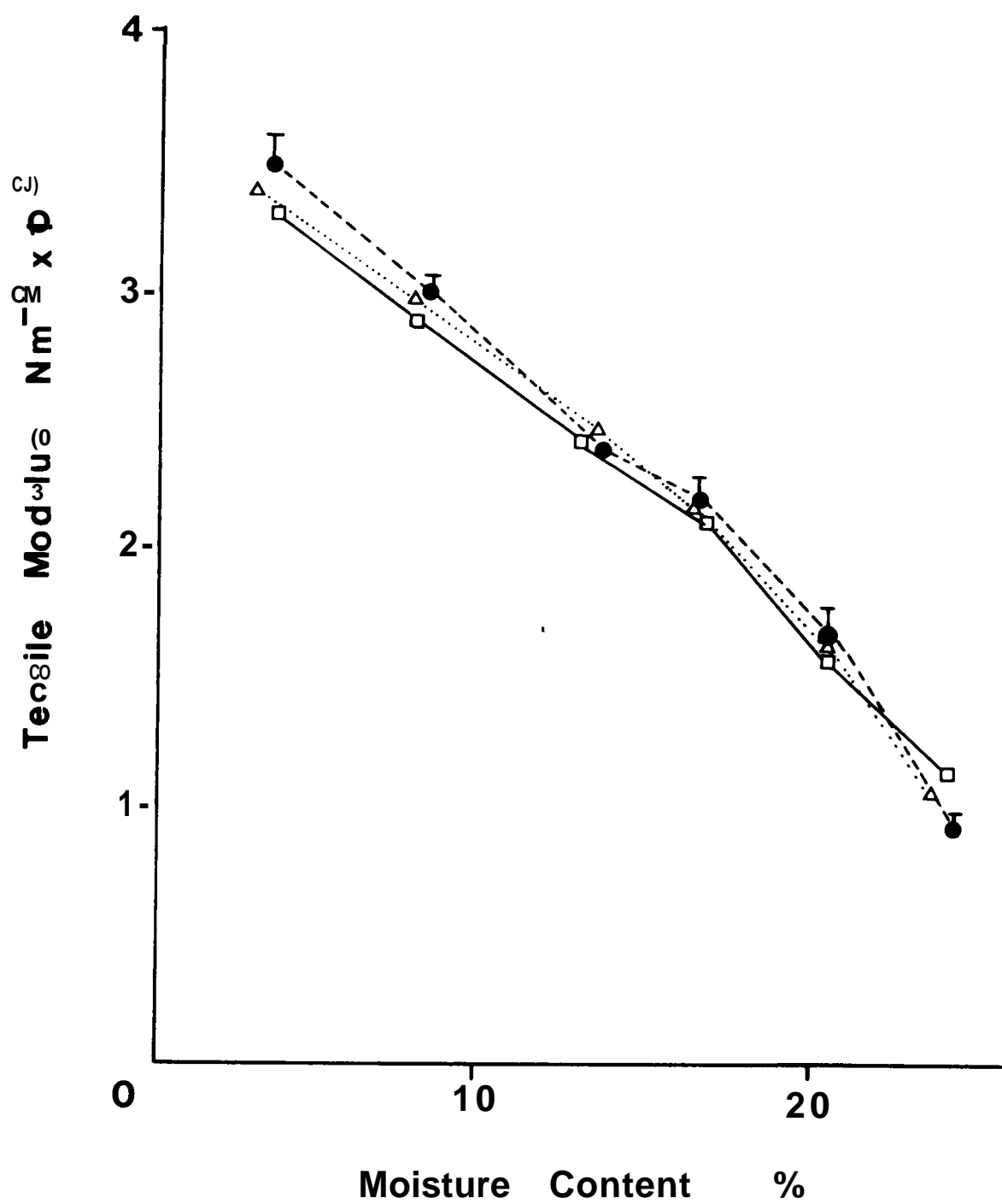
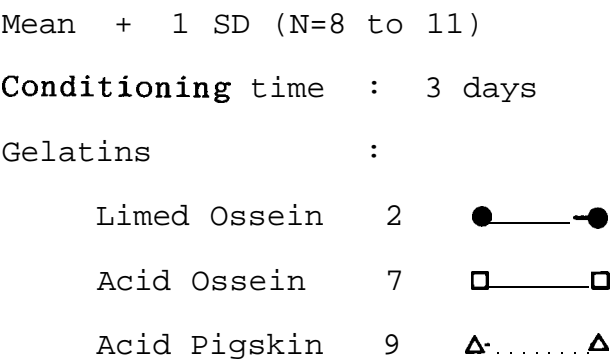


FIGURE 6.9

The Variation of Fracture Stress with Moisture Content



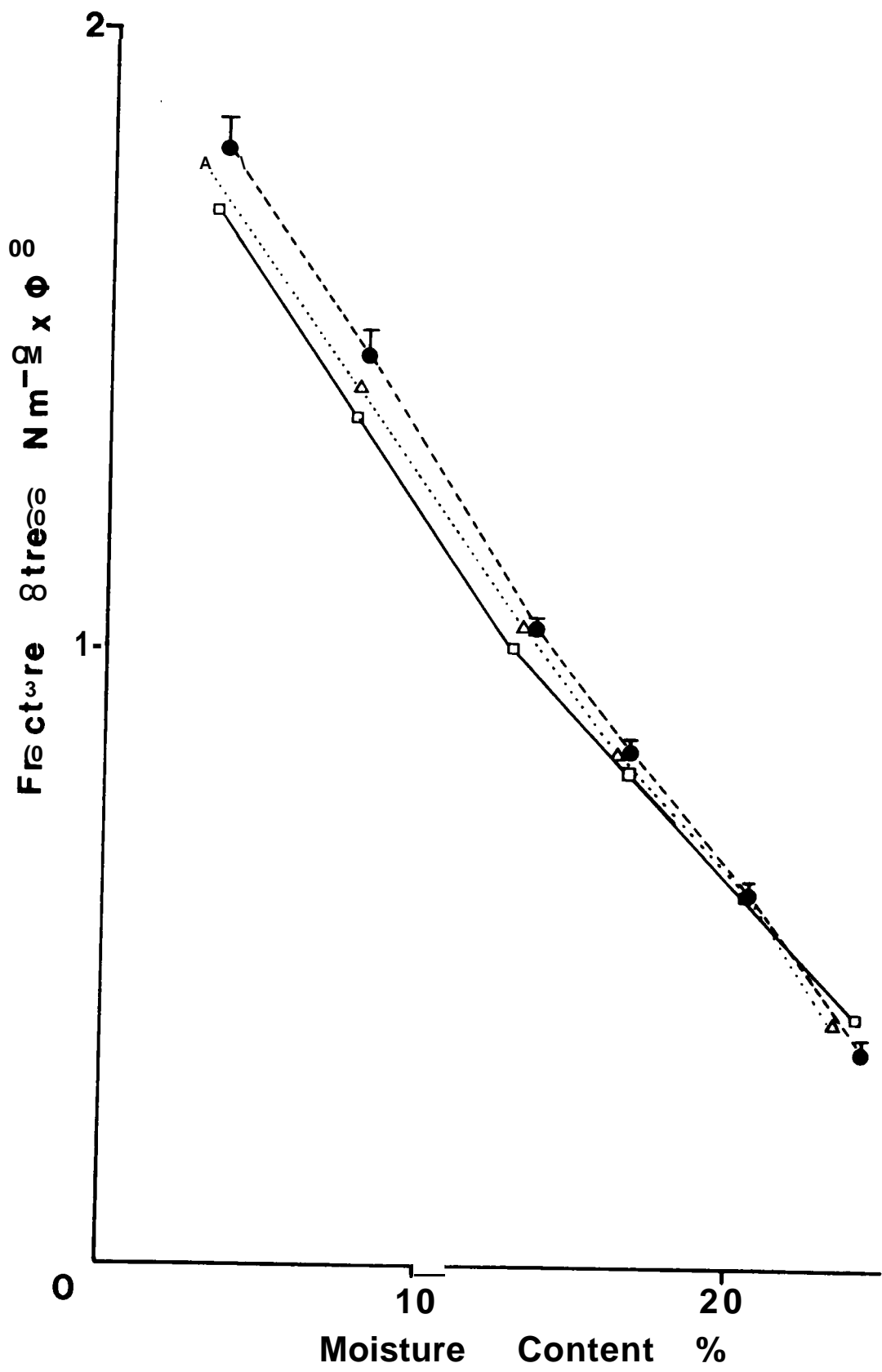


FIGURE 6.10

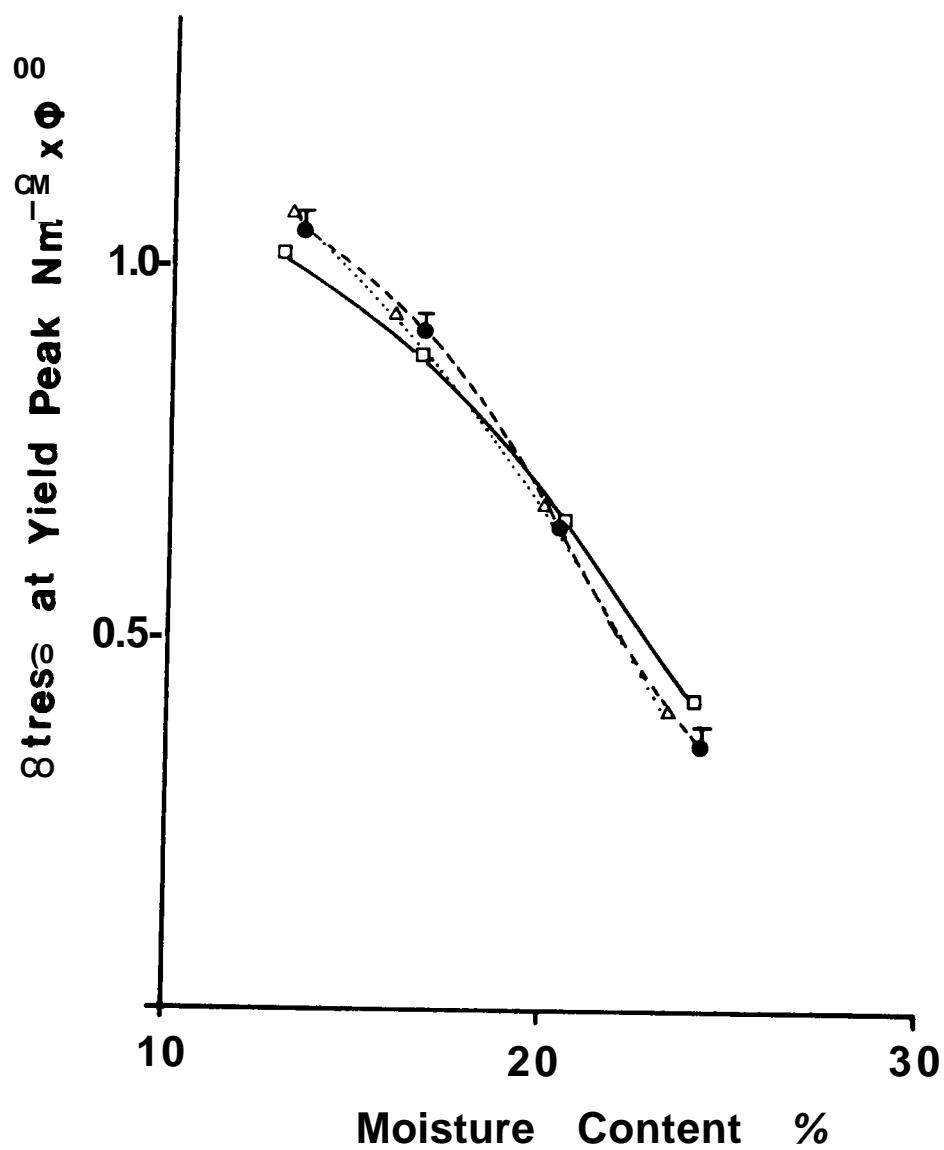
The Variation of Yield Stress with Moisture Content

Mean + 1 SD (N=8 to 11)

Conditioning time : 3 days

Gelatins :

Limed Ossein	2	●——●
Acid Ossein	7	□——□
Acid Pigskin	9	Δ.....Δ



FSTN values had large standard deviations ; up to 20% of the mean value (Fig.6.11). Acid ossein and pigskin batches did **not** differ but those of the limed ossein batch were greater at all moisture contents and showed a maximum at 13% moisture. TM, FSTS and YSTS values being similar, this indicated the limed ossein possessed greater fracture toughness at a moisture content corresponding to 33% R_H . This is within the range of 30 - 40% R_H cited as optimum for the storage and filling of hard capsules (46,47). The entire range of gelatins were subsequently tested at this humidity.

6.3,2,5 The Effect of Gelatin Type

Table 6,12 shows the stress-strain characteristics for unblended gelatin films over the entire range of gelatin batches conditioned at 33% R_H for 3 days,

TM values varied over a range of $0,15 \text{ Nm}^{-2} \times 10^9$; 6% of the overall **mean**. Likewise FSTS and YSTS varied by only 5%, It was considered that these differences were of limited practical significance although it is of interest to note the highest values of FSTS were those of the 'good' limed ossein and pigskin batches, FSTN values varied by more than 45% and this data was subjected to statistical **analysis**.

The significant **max:minimum** variance ratio (f = **8.2**: $p < 0.01$) demanded a non-parametric test for the

FIGURE 6.11

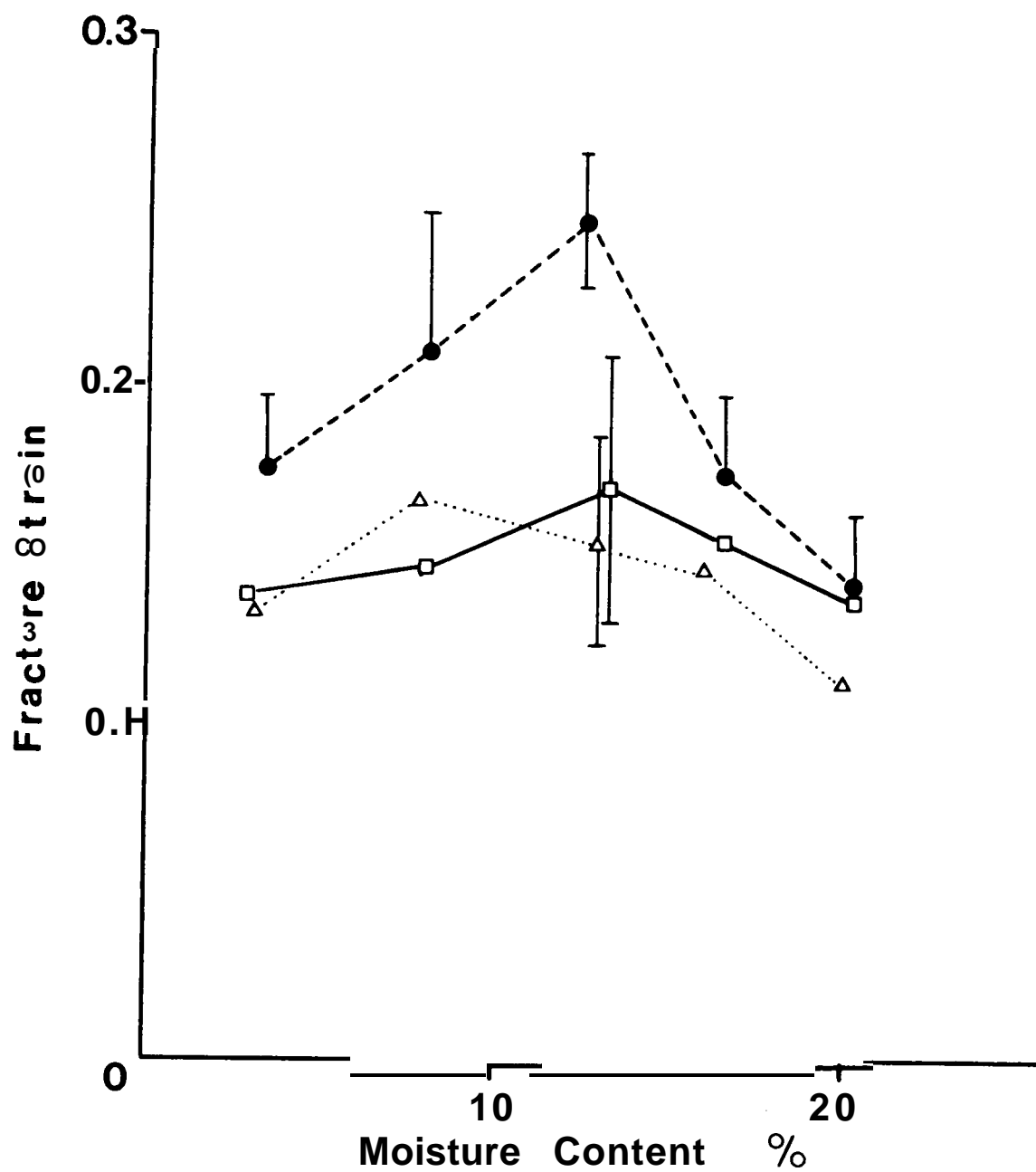
The Variation of Fracture Strain with Moisture Content

Mean + 1 SD (N=8 to 11)

Conditioning time : 3 days

Gelatins :

Limed Ossein	2	●—●
Acid Ossein	7	□—□
Acid Pigskin	9	Δ....Δ



G elatin Tpe	Batch	Tensile Modulus		Stress at Yield		Fracture Stress		Fracture strain	
		Mean	SD	peak Nm ⁻² x 10 ⁸	SD	Mean	SD	Mean	SD
Lived Oseei (good)	2	2.322	0.038	1.040	0.008	1.088	0.018	0.246	0.020
	3	2.398	0.040	1.061	0.012	1.080	0.009	0.217	0.014
Lived Oseei (poor)	4	2.392	0.041	1.015	0.016	1.018	0.021	0.200	0.031
	5	2.416	0.075	1.034	0.013	1.008	0.025	0.209	0.017
Acid Oseei	6	2.469	0.15	1.042	0.012	0.984	0.024	0.157	0.022
	7	2.447	0.060	1.033	0.012	1.017	0.024	0.162	0.016
Acid Pigskin	8	2.388	0.028	1.018	0.009	1.007	0.022	0.175	0.019
	9	2.471	0.53	1.067	0.16	1.087	0.027	0.168	0.040

TABLE 6.12 The Stress-Strain Characteristics of Unbleached Gelatin

N = 80 ± 1 Strain rate = 1 x 10⁻² s⁻¹.

Conditioned 8 days at 88% RH, 18 ± 2°C.

comparison of gelatin batches. Two-tailed Mann Whitney U-tests at $p = 0.01$ were performed between pairs of **batches**. Figure 6.13 shows the results of these tests and the information is rearranged for illustrative purposes in Figure 6,14. Horizontal lines link batches not **significantly** different e.g, batch 4 is not different from batches 5, 8 and 9,

The FSTN value of 2 is **significantly** higher than all others, 9 has a large degree of scatter and cannot be shown different to any other batch, 6, 7 and 8 form a distinct subgroup as do 3 and 5, 4 has elements in common with both subgroups.

The groupings are not along lines of gelatin type and hence we can make no generalizations concerning the relation of fracture toughness to **type**. Batch 2 appears to possess an exceptionally high toughness,

6,3,2,6 -- The Effect of Blending

Electrostatic interaction occurs in dilute solution between limed and acid gelatins (192,193) and synergistic increases in rigidity modulus have been reported for gels of 5 - 50% concentration containing blends of limed and acid osseins, an effect that increases with increasing total concentration, (185,186),

Capsules are manufactured from a blend of limed and acid gelatins and therefore experiments were conducted to investigate the effects of blending on film mechanical properties.

FIGURE 6.13

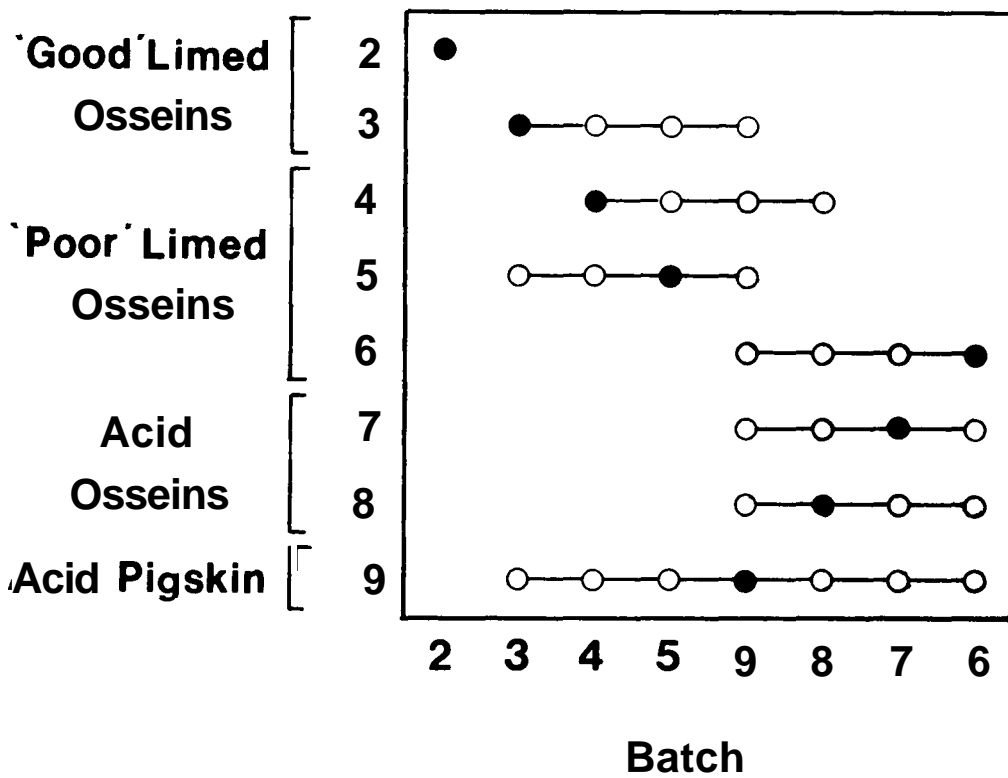
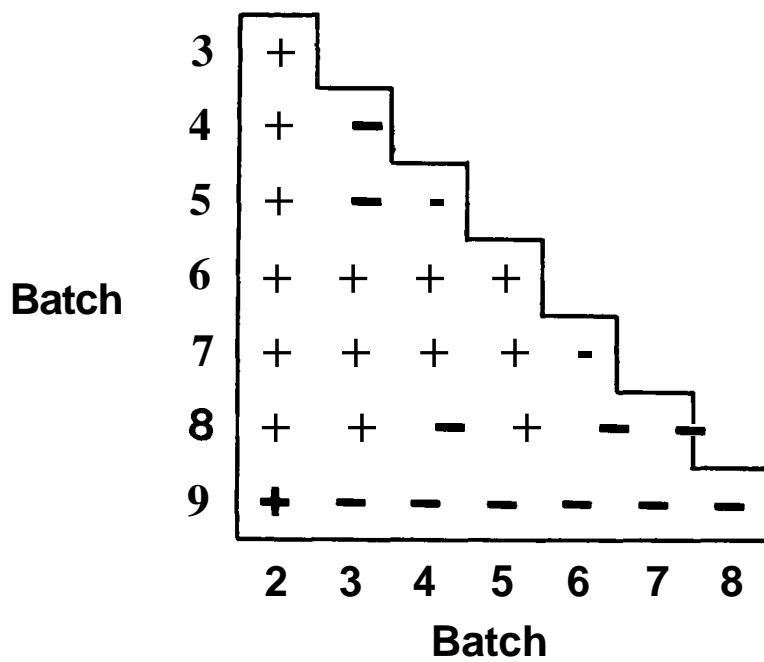
The Results of Paired Mann-Whitney U-tests between the
FSTN values of Gelatin Batches

+ indicates a significant ($p < 0.01$) difference
between the batches indicated.

FIGURE 6.14

A Diagram showing the Grouping of Gelatin Batches not
Significantly different from each other

The horizontal lines connect batches not
significantly different.



A limed and an acid ossein were blended in varying proportions and the films were tested after conditioning for 3 days at 33% R_H . The results (Fig. 6.15) show that blending has no effect on TM, FSTS and YSTS. Figure 6.16 indicates that the addition of 20% acid ossein gelatin has a disproportionate effect in lowering FRSTN. At no time is the FRSTN value higher than that of the unblended gelatins.

6.3.2.7 Blends containing 'Good' and 'Poor' Limed Ossein Gelatins.

In the industrial process, gelatins are blended 5:1 , Limed : acid ossein (section 1.3.1) and the possibility exists that 'poor' limed ossein batches become apparent only when blended. All batches of limed ossein were blended 5 : 1 (83% w/w limed ossein) with an acid ossein batch and the films tested after 3 days at 33% R_H . In addition, a film in which 10% of batch 2 blend had been replaced by batch 10 was also tested. 6.17 shows the stress-strain characteristics of these films and figure 6.18 a statistical analysis of FSTN values by the method of section 6.3.2.5.

Gelatins cannot be divided into **significantly** different FSTN subgroups on a basis of 'good' or 'poor' suitability for capsule manufacture. Batch 6 has the lowest FSTN value consistent with the results on unblended films (6.3.2.5) where it is grouped with acid osseins. The addition of 10% low-bloom hide gelatin does not depreciate any stress-strain parameter.

FIGURE 6.15

The Effect of Blending a Limed and an Acid Ossein Gelatin
on The Tensile Modulus, Yield Stress and Fracture Stress
of Gelatin Films

Mean \pm 1 SD (N=8 to 11)

Gelatin batches 2 and 7

Conditioning Time : 3 days at 33% R_H

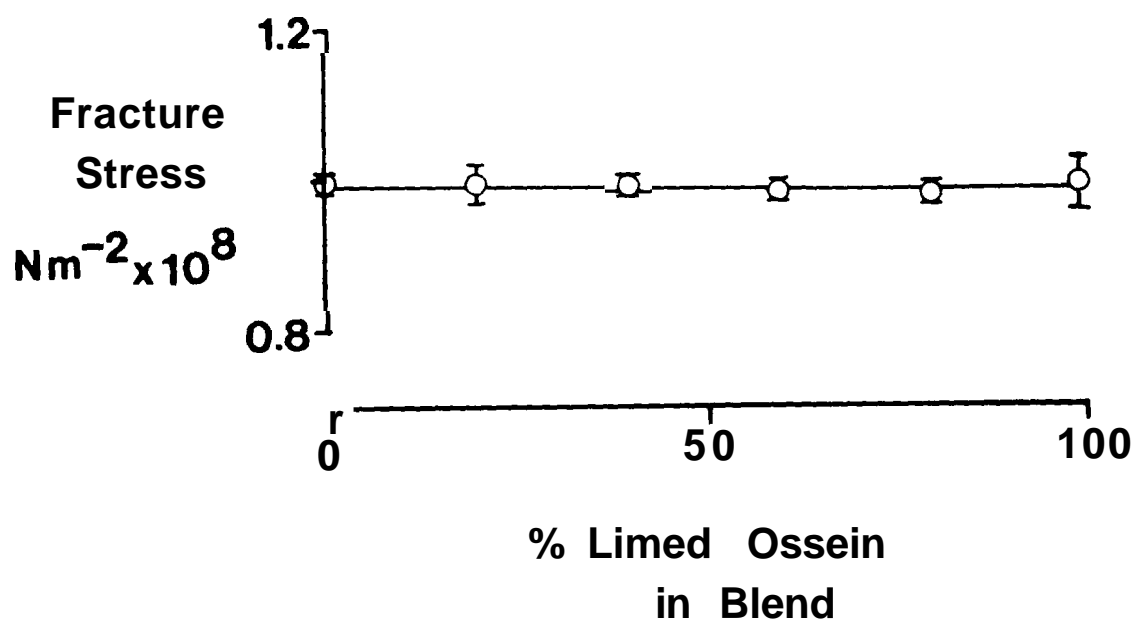
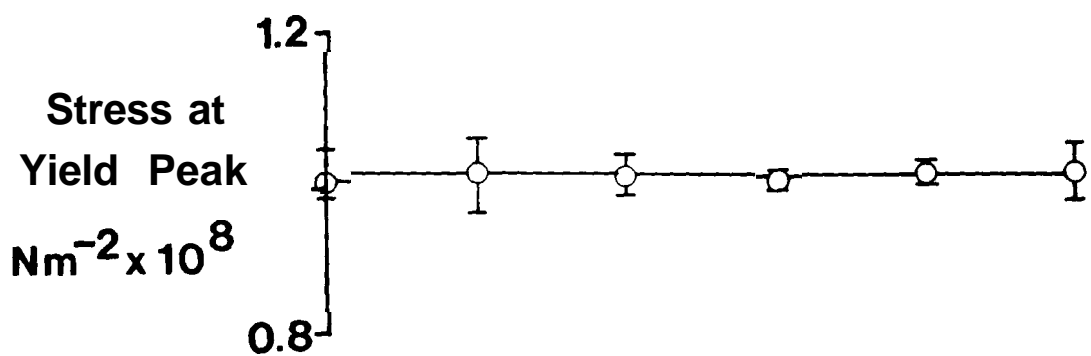
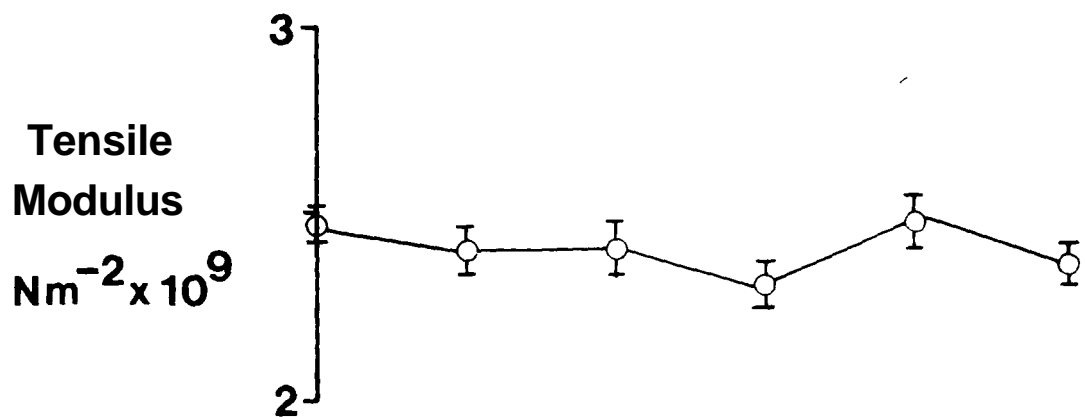


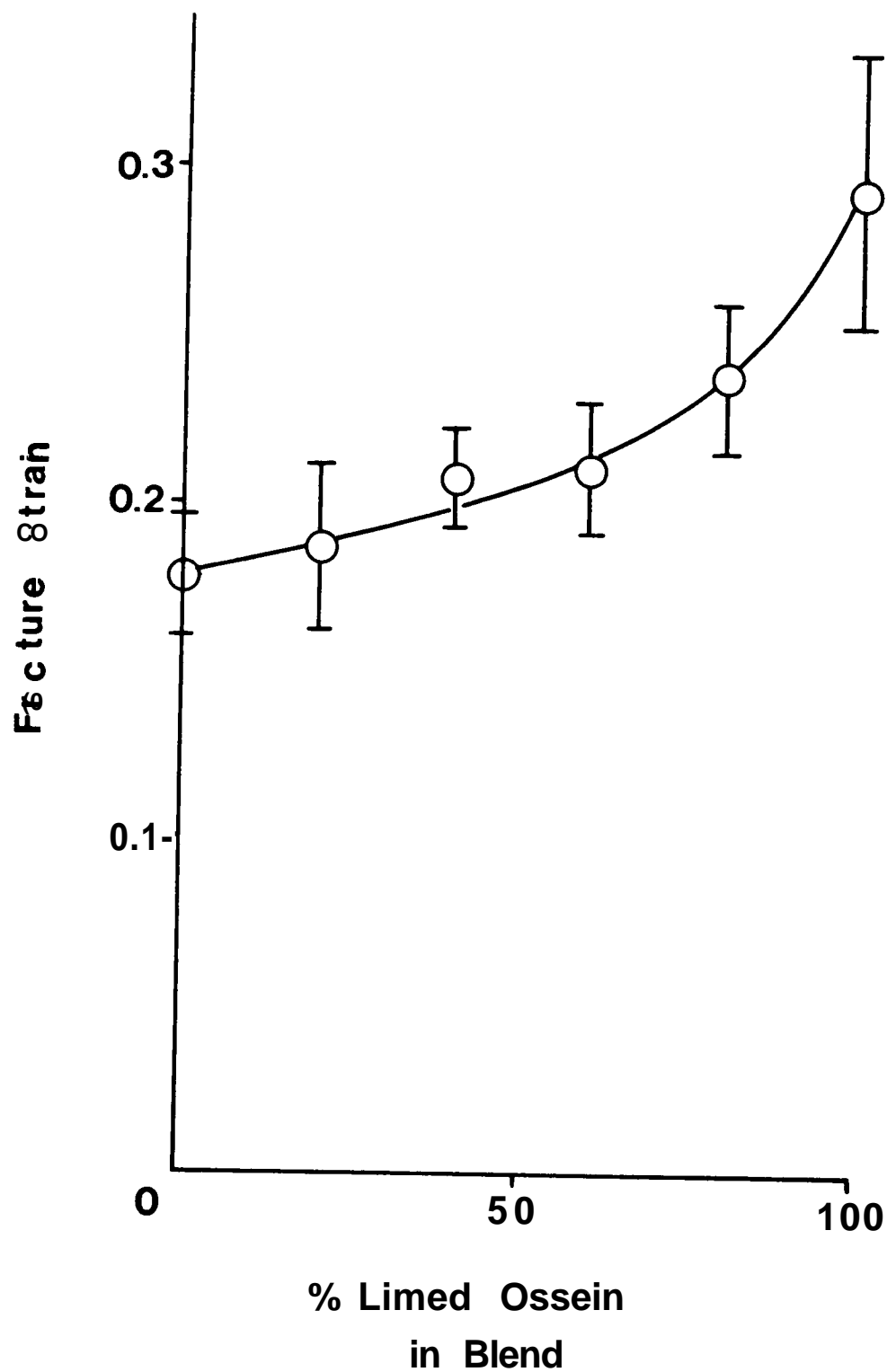
FIGURE 6.16

The Effect of Blending a Limed and an Acid Ossein Gelatin
on Fracture Strain

Mean \pm 1 SD (N=8 to 11)

Gelatin batches 2 and 7

Conditioning Time : 3 days at 33% R_H



Geiati= Etype	B ^a rc ^a	Mo=8i ^o Mo du ^u 8 No-2 x ^o 09	Str ^o 88 a ^o Yi ^o ld P=a ^x N ^m = x ^o 108	ca i ^u ro Stres8 mN-2 x ^o 108	Str ^o 88 M=a ^o	Str ^o 88 M=a ^o	Str ^o 88 M=a ^o
		SD	SD	SD	SD	SD	SD
Li ^o do 88 ^o in (good)	2	2 .422	0.068	1.070	0.009	1059	0.019
	2A	2 .422	0.078	1.102	0.018	1050	0.010
	3	2 .042	0.072	1.087	0.014	1058	0.015
Li ^o do 88 ^o i= (poor)	4	2 .408	0.065	1.070	0.011	1058	0.012
	5	2 .442	0.050	1.092	0.011	1038	0.011
	6	2 .480	0.042	1.100	0.024	1072	0.016

TA BLE 8.17

Tr^oo Str^o88-Strai= Chara^oristics of Li^odo 88^oi= Gei^oati=8

8i^odo=do d 5:1 wi^ox A o i^odo 88^oi= Gei^oati=8

N = 2 to 11. Str^o88i= 22^oo 1 x 10⁻²g⁻¹.

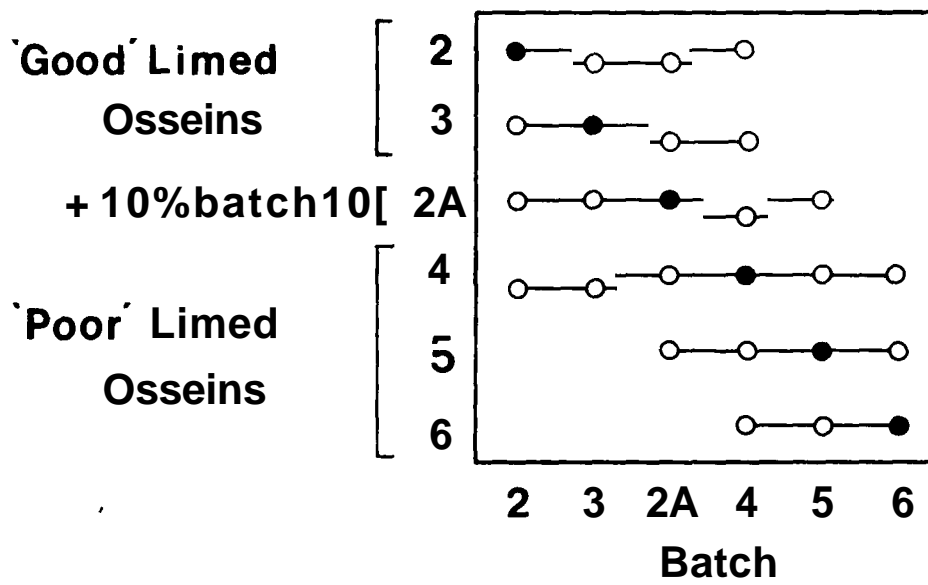
Co=di^oio=do d 8 day 8 a^o 22% R_H, 2 ± 2 °C.

22^ooC^o 22^o is = 8a^och = bl^ood with 10% 22^och 10 add^od.

FIGURE 6,18

The Grouping of Gelatin Batches with FSTN Values not
Significantly Different at $p = 0,01$

The horizontal lines connect batches not
significantly different.



6.4 STRESS-RELAXATION EXPERIMENTS

6.4.1 Method

Dry film strips were prepared, conditioned and mounted as before (section 6.3.1). Sample dimensions were as 6.3.1 but 10mm wide and of gauge length 70mm. A strain of 0.01 (within the linear viscoelastic region) was applied at a rate of $1 \times 10^{-2} \text{ s}^{-1}$, and measured from the induced stress and previously determined TM values. The 60-second stress relaxation modulus $E_r(60)$ was calculated from the **stress:strain** ratio after 60 seconds.

6.4.2 Results and Discussion

A typical stress relaxation curve (after conditioning at 33% R_H for 3 days) is shown in Figure 6.19. The overall decrease in load was of the order of 1.3N after 60 seconds. This necessitated the use of a pre-set zero-suppression and a change in instrument sensitivity.

Table 6.20 shows values of $E_r(60)$ for unblended films conditioned 3 days at 33% R_H . The range of means is no more than $0.14 \text{ Nm}^{-2} \times 10^9$ (6% of the overall mean) and reduces to 2% if a mean TM value is used to calculate the applied strain. This is within experimental error and also indicates that much of the scatter may be ascribed to variation in the original TM determinations. Likewise $E_r(60)$ values for 0 - 100% blends (**Fig.6.21**) and 5:1 blends (**Fig.6.22**) are smoothed by using a pooled TM value. The range of 2% in this mechanical property is **insignificant** in relation to the errors of the experiment and assumes no practical importance.

FIGURE 6.19

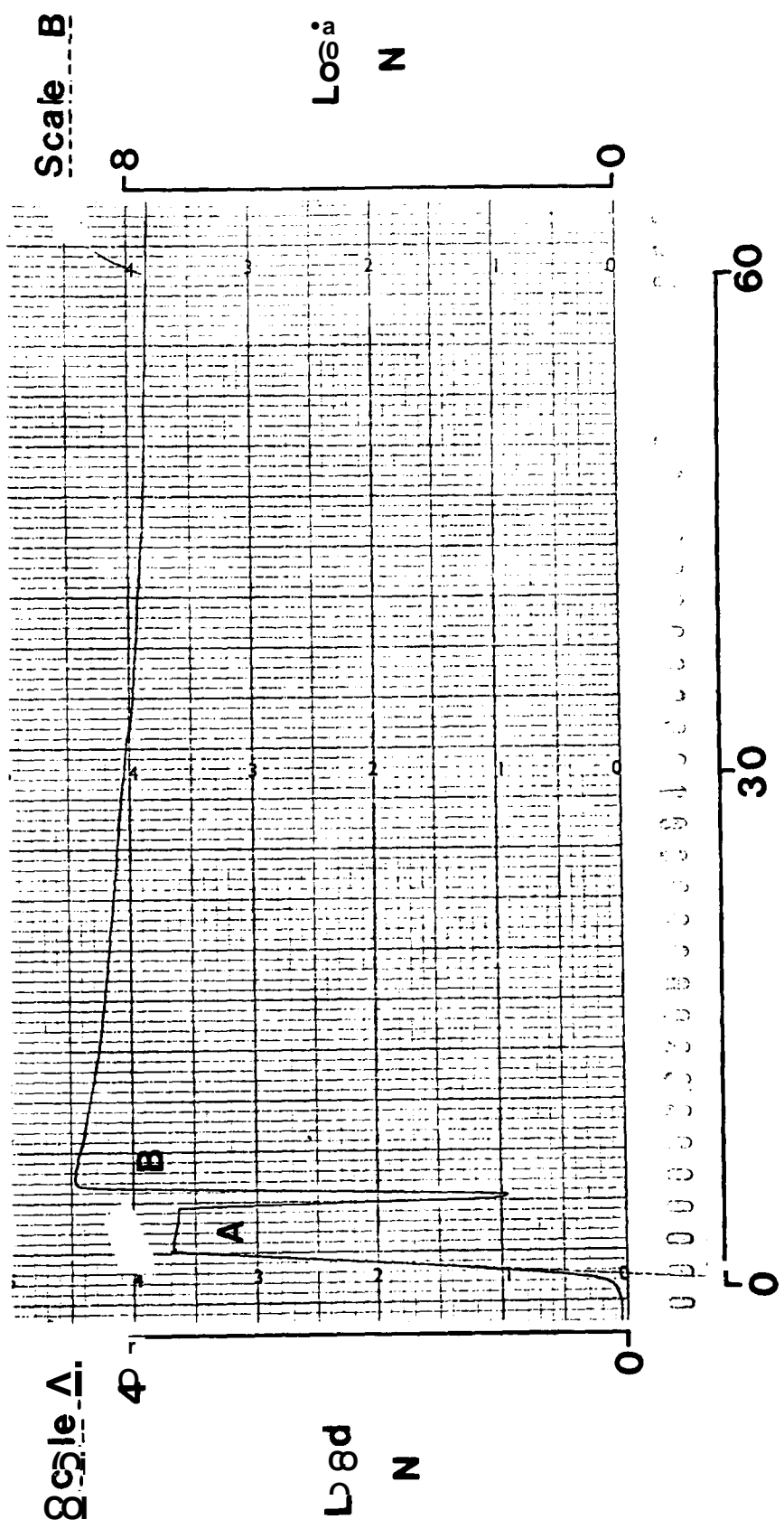
A Typical Stress-relaxation profile

Gelatin Batch 8

Conditioned 33% R_H for 3 days

Scale A is for the initial loading curve (A)

Scale B is for part B of the trace after a zero
suppression of **27.7N** and a five-fold sensitivity
increase.



G o l s t i n p °	S a t c x	8 v r ° 88 R ° i e x p ° i o = M o d u l i n 38 (E r 80) M ° - ° x 10 ⁹			
		8 v r ° i =	from i = d i v i d u l E M v a l u e s	8 t r i o f r o m a w o g T M v a l u e	
		M ° o =	SD	M ° g =	S D
'Good' L i m ° d O 88 ° i = 8	2	C C 29	0 . 0 12	2 2 199	0 . 0 11
	3	M C 12	0 . 0 17	2 3 08	0 . 0 17
'Poor' L i m ° d O 88 ° i = 8	4	C C 98	0 . 0 12	2 3 00	0 . 0 11
	5	M C 12	0 . 0 08	2 2 91	0 . 0 07
A o d O 88 ° i = 8	6	C C 22	0 . 0 22	2 2 64	0 . 0 22
	7	M C 95	0 . 0 06	2 2 85	0 . 0 06
A c i s P i g 8 k i =	8	C C 11	0 . 0 10	2 3 18	0 . 0 10
	9	M C 67	0 . 0 07	2 2 93	0 . 0 07

TA 8 EL 8 2. 0

V a l u e s o f 8 ° - 88 ° C O = 3 8 v r ° 88 R ° i e x p ° i o = M o d u l i n 38 f o r

U = X l o = 303 G o i n g i n f i l m 8

N = 5 ° o 7. 8 v r i p 8 C O = 3 i v i s = 03 8 d e y 8 o ° 88% R_H.

FIGURE 6.21

The Effect of Blending a Limed and an Acid Ossein Gelatin
on the 60-Second Stress Relaxation Modulus

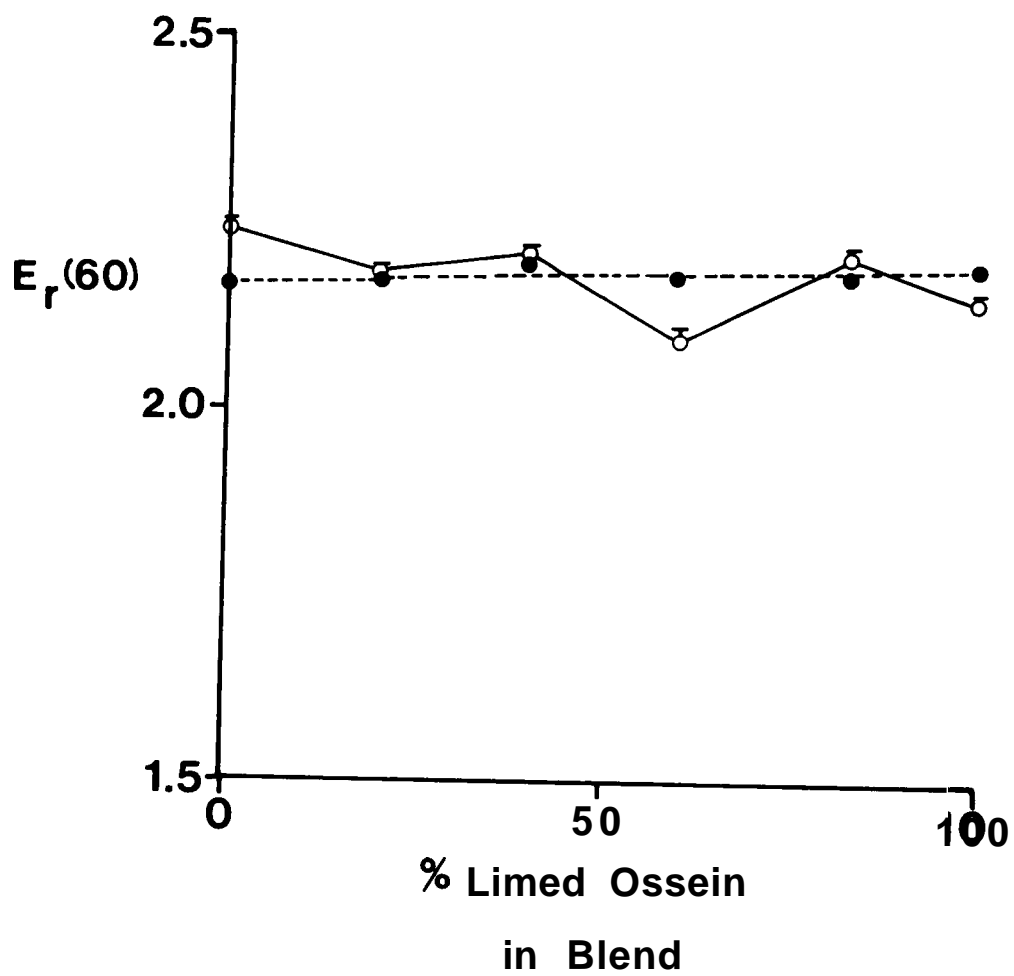
Gelatin Batches 2 and 7 blended

Mean + 1 SD (N=5 to 7)

Strain calculated

○—○ From individual TM values

●—● From a mean TM of $2.39 \text{ Nm} \times 10^{-2}$ ⁹



Gelatin	Batch	E _r 60	
		Mean	SD
Limed Ossein (good)	2	2.245	0.026
	3	2.228	0.008
	2A	2.268	0.010
Limed Ossein (poor)	4	2.241	0.022
	5	2.221	0.013
	6	2.241	0.009

Table 6.22 Values of 60-second Stress Relaxation
Modulus (E_r (60)) for Limed Ossein Gelatins
Blended 5:1 with Acid Ossein Batch 7

N = 5 to 7

Conditioned 3 days at 33% R_H. 18 ± 2°C.

e calculated from a mean TM of 2.428 Nm⁻² x 10⁹

6.5 THE OPTICAL ROTATION OF GELATIN FILMS

6.5.1 Introduction

Sung (23) has concluded that optical rotation measurements provide a sensitive indication of helical content in gelatin films. The strong **laevorotation** originates not from the triple helix structure itself but from the intramolecular helical conformation of the polypeptide chains held within it (74). The high specific rotation of films when compared to gels, has been shown to be due not to high helical content but to **the** orientation of helices in the plane of the film as a consequence of **unidirectional** shrinkage during drying (24-26, 33). Coopes has shown that the helices lie at an average of 18° to the plane of the film for a 2 mm thick 5% gel dried to **90%** gelatin content (26). The planarity of the helices increases for films cast from more dilute solutions (60).

Other factors affect the optical rotatory power of cold-dried gelatin **films**. The gelling and drying temperature affects the nucleation **and** growth of helical areas (**60,166,193**) and drying rate and film thickness determine the degree of helical growth attainable before molecular movement is "frozen" by evaporation of the solvent. Very rapidly-dried thin films have a lower specific rotation than slow-dried thicker ones (27).

Birefringence from strain, local molecular orientation, foreign bodies and blemishes must be avoided for reliable results (59).

The following experiment aims to complement the results of mechanical testing using optical rotation measurements as an indicator of film helicity.

6.5.2 Method

Films were prepared and slow-dried under cover in the HTCR as described in section 2.2. They were conditioned at 20°C 33% R_H for 3 days.

An NPL Automatic Polarimeter type 143C (a) fitted with a sodium filter (589nm), four-place digital readout and calibrated with sucrose (b) solution (194) was used for the measurements. Unmarked, flat pieces of film, of thickness 0.16 - 0.18 mm, were placed on top of the sample cell and held by a metal ring. Readings were offscale and were brought within the range of the instrument by filling the 1 cm sample cell beneath with 12% w/w sucrose solution, the rotation of which had been previously **determined**. Film rotation readings were taken and repeated after rotating the sample 90° to check for **birefringence**. Discrepancies of more than 0.01 degrees (1%) resulted in rejection of the sample. The specific rotation was calculated from the equation

$$[\alpha]_D^{20} = \frac{\alpha}{1 \cdot d}$$

-
- (a) Thorn Automation Ltd., Basford, Nottingham, UK.
 - (b) Analar Grade, BDH Chemicals Ltd., Poole, UK.

$[\alpha]_D^{20}$:	specific rotation at 589nm and 20°C
a	:	measured rotation (degrees)
l	:	thickness (dm)
d	:	weight ₃ of gelatin per unit volume (g.cm ⁻³)

l was measured with a micrometer and d was determined from pycnometric volume measurements (c) and film moisture determinations. Eight replicate determinations were performed for each **result**.

6.5.3 Results

Over the range 0.16 - 0.18 mm $[\alpha]_D^{20}$ did not appear sensitive to film thickness (Fig. 6.23). The values are consistent with data on slow-dried films presented by Coopes (166).

Table 6.24 shows $[\alpha]_D^{20}$ values for each gelatin film. The quoted deviations are pooled from the determinations of optical rotation, volume and moisture content by the method of Topping (195). The results of a Mann Whitney U-test, carried out as in section 6.3.2.5 are shown in Table 6.25.

Acid gelatins possessed **significantly** higher $[\alpha]_D^{20}$ values than limed gelatins. One limed ossein (Batch 3) possessed a **significantly** lower rotation than any other batch. A blend of 50 : 50 limed : acid ossein gelatins had a rotatory power no greater than that of the limed osseins.

(c) Air Pycnometer, Beckmann **Ind.**, Irvine, C.A., USA.

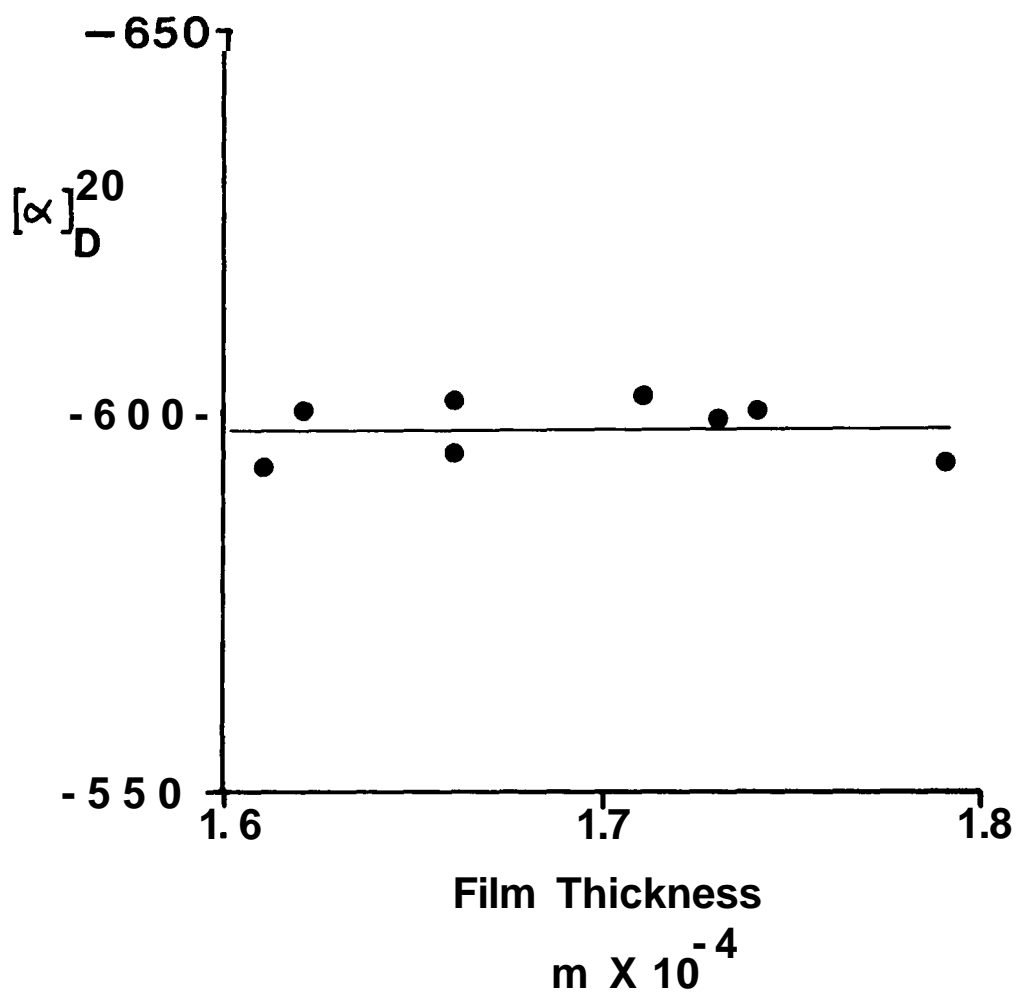
FIGURE 6.23

The Relation between Film Thickness and Specific Rotation

$[\alpha]_D^{20}$

Gelatin Batch 3

Conditioned 3 days at 33% R_H



Gelatin Type	Batch	Mean Film Thickness m x 10 ⁻⁴	$[\alpha]_D^{20}$ (degrees')	
			Mean	SD
'Good' Limed Ossein	2	1.75	-613.9	4.8
	3	1.69	-596.0	10.7
'Poor' Limed Ossein	4	1.68	--616.8	4.7
	5	1.71	--612.1	9.0
	6	1.68	--611.2	7.4
Acid Ossein	7	1.72	--639.6	8.9
	8	1.71	--624.8	9.6
Acid Pigskin	9	1.67	-630.2	8.2
Blend 50:50 of batches 2 & 7		1.75	-613.2	4.4

~~Table 6.24~~ ~~The Specific Rotation Values $[\alpha]_D^{20}$ of~~
Gelatin Films (N=6 to 8)

Group	Batches
1	7
2	8, 9
3	2, 4, 5, 6, 11
4	3

Table 6.25 Groups of Gelatins **significantly** different
at p = 0.01 by the Mann-Whitney U-test

CHAPTER VII

CHAPTER VII

THE FRICTIONAL PROPERTIES OF GELATIN FILMS

7.1 INTRODUCTION

Friction is the resisting force that arises when the surface of a body slides over an adjoining surface (143). It is expressed as the coefficient of friction (μ) which is the ratio of the frictional force (F) to the force acting normal to the two surfaces (W).

$$\mu = \frac{F}{W}$$

The static coefficient of friction μ_s relates to the force required to initiate movement, whilst the kinetic coefficient μ_k describes the force required to sustain motion at a given sliding velocity. In general, $\mu_s > \mu_k$. The classical laws of friction (148) state that frictional **force is:**

- 1) Independent of the area of contact for a constant normal load.
- 2) Proportional to the normal load (i.e. μ is independent of load),
- 3) Independent of sliding velocity.

Friction is a complex summation of many factors arising from the contact of microscopic surface irregularities (asperities)

The entire normal load is carried by the tips of the asperities which consequently suffer severe deformation and are brought into intimate atomic contact. Over these regions adhesion occurs which, in thermoplastic polymers, is mediated by van der Waal's Forces or hydrogen bonding (146). Movement requires the dissipation of energy in shearing these junctions either at the plane of contact or within the material nearby (144-146). For materials that irrecoverably, plastically deform such as metals, the true contact area is proportional to the normal load and law 2 is a good approximation. However, with rigid polymers there is a significant contribution from elastic deformation and μ obeys a law of the form

$$\mu = k W^{x-1} \quad \text{where } 0.67 < x < 1 \quad (144,147).$$

The self-sliding friction of rigid polymers is affected by many other factors. Temperature, strain rate (sliding velocity) and humidity alter viscoelastic properties thereby altering μ and a fast sliding rate will generate heat (149). Molecular orientation may give rise to frictional anisotropy (150,151) "Slip-stick" motion may occur, and is attributed to μ_s being greater than μ_k (152,153). Sliding may generate static charges (149). The age of the film may determine the degree to which blooming agents (slip additives) may have been extruded from the matrix onto the surface thereby reducing friction (143)

Finally, measurements of friction are notoriously variable (153) and are highly dependent on the condition and cleanliness of the two surfaces.

7.2 APPARATUS

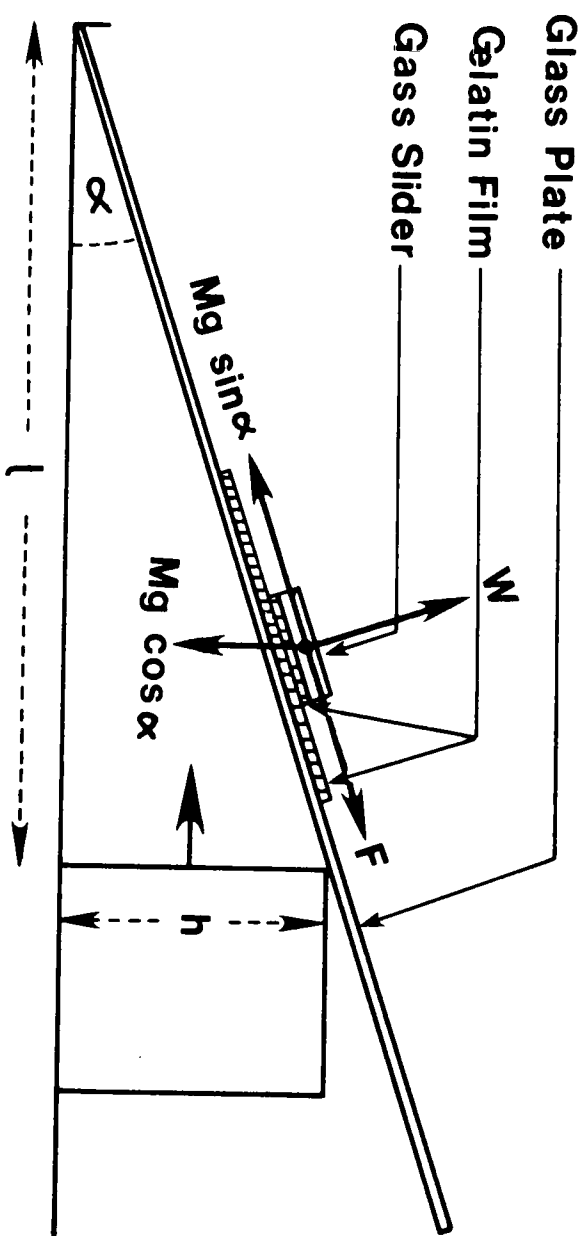
The apparatus for the determination of static coefficient of friction is shown in Figure 7.1 and apart from the size of slider, was of the same design as that used for the determination of "limit gliding angle" in the gelatin industry (40). It consisted of a smooth levelled base upon which a large glass plate was raised by uniform movement of a wood block underneath. The slider consisted of a **gelatin-faced** microscope slide of dimensions 75 x 25 mm which was placed transversely upon a piece of film stuck to the large glass plate.

7.3 METHOD

Dry films were prepared as section 2.2 on clean, unmarked, **grease-free, spreading** plates. The surface of the film formed against the glass was used for the sliding surfaces. A piece of film cut to size with a guillotine was stuck onto the slider with double-sided tape. Likewise a piece of 50 x 120 mm was stuck transversely across the glass plate. Each surface was examined for flatness with reflected light and samples with damaged or curled edges, or with surface marks were discarded. The gelatin film was handled throughout with gloves. The slider was placed upon the lower film and checked for freedom of movement.

FIGURE 7.1

A Schematic Diagram of the Apparatus used for the
Determination of the Static Coefficient of Friction
of Gelatin Films



The glass plate was then lifted by smoothly propelling the wood block along the base at approximately 20 mm.s^{-1} until slip occurred. Readings were rejected if the slippage was uneven, where 'blocking' occurred (adhesion due to raised areas of film) or where slip was initiated by jerky movement.

The static coefficient of friction μ_s was calculated as the ratio $\frac{h}{l}$, derived as follows (see Figure 7.1),

$$\mu_s = \frac{F}{W} = \frac{Mg \sin \alpha}{Mg \cos \alpha} = \tan \alpha = \frac{h}{l}$$

where

F : frictional force

W : normal load

M : mass of slider

g : acceleration due to gravity

a : angle of repose

h : height of wood block

l : distance along the base of wood block from the hinge.

Experiments were carried out in the humidity and temperature controlled room described in section 2.4.

7.4 RESULTS AND DISCUSSION

7.4.1 The Variability of Repeated Determinations

Repeated determinations on the same sample showed considerable scatter of results (Figure 7.2) attributable

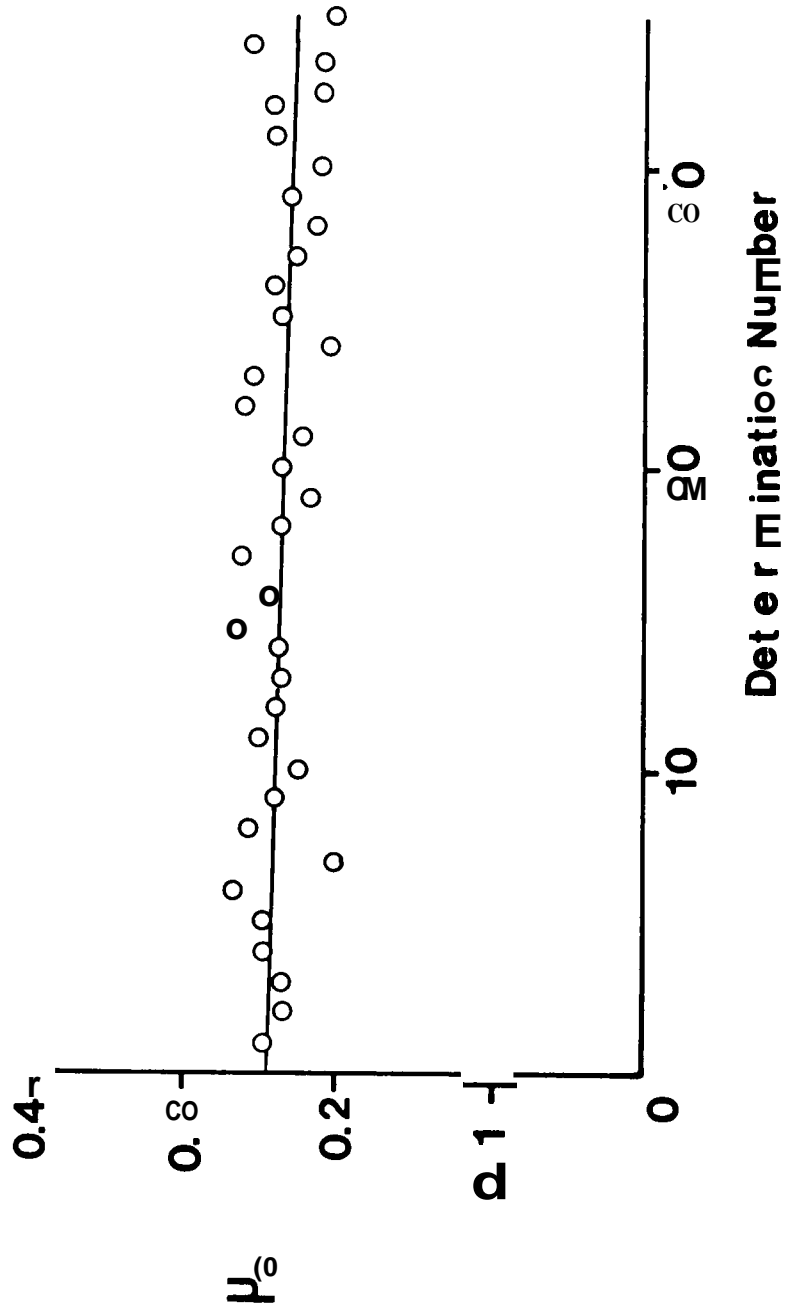
FIGURE 7.2

The Coefficient of Friction (μ_s) of Repeated Determinations
on the same Film Sample

Gelatin Batch 3

Film conditioned at 33% R_H , 20°C for 3 days

Normal Load 0.1 1N



to the variation of friction at different points of the film (154). The use of an anti-static device* did not reduce the scatter. A mean of 5 results was taken for each sample, this being more reliable than a single reading. The small negative slope of a line fitted by linear regression through the data may be explained in terms of surface wear or static accumulation. The value of 0.25 for gelatin film conditioned at 20°C and 33% R_H for three days is within the range for the self-sliding of many synthetic polymer surfaces (148,153,155) although μ_s values are highly dependent on individual preparative conditions.

7.4.2 The Effect of Normal Load

In common with many rigid polymers μ_s decreased with increasing normal load (Figure 7.3) (153,155). This is a consequence of the elastic contribution to asperity deformation described earlier. Normal load was standardized at 1.1×10^{-7} N in subsequent experiments.

7.4.3 The Effect of Humidity

Film samples were conditioned and tested between 29 - 52% R_H ; the range of the humidity and temperature controlled room (HCTT). μ_s increased from 0.2 to 0.4 over this humidity range (Figure 7.4). The lowering of modulus and yield stress with increasing moisture content would lead to a greater asperity contact area and thereby an

* Anti-Static Gun, Zerostat Components PLC, St.Ives, Cambs, U.K.

FIGURE 7.3

The Variation of Coefficient of Friction (μ_s) with
Normal Load

Gelatin Batch 3

Film conditioned at 33% R_H , 20°C for 3 days

Mean \pm 1 std.dev.(n=6)

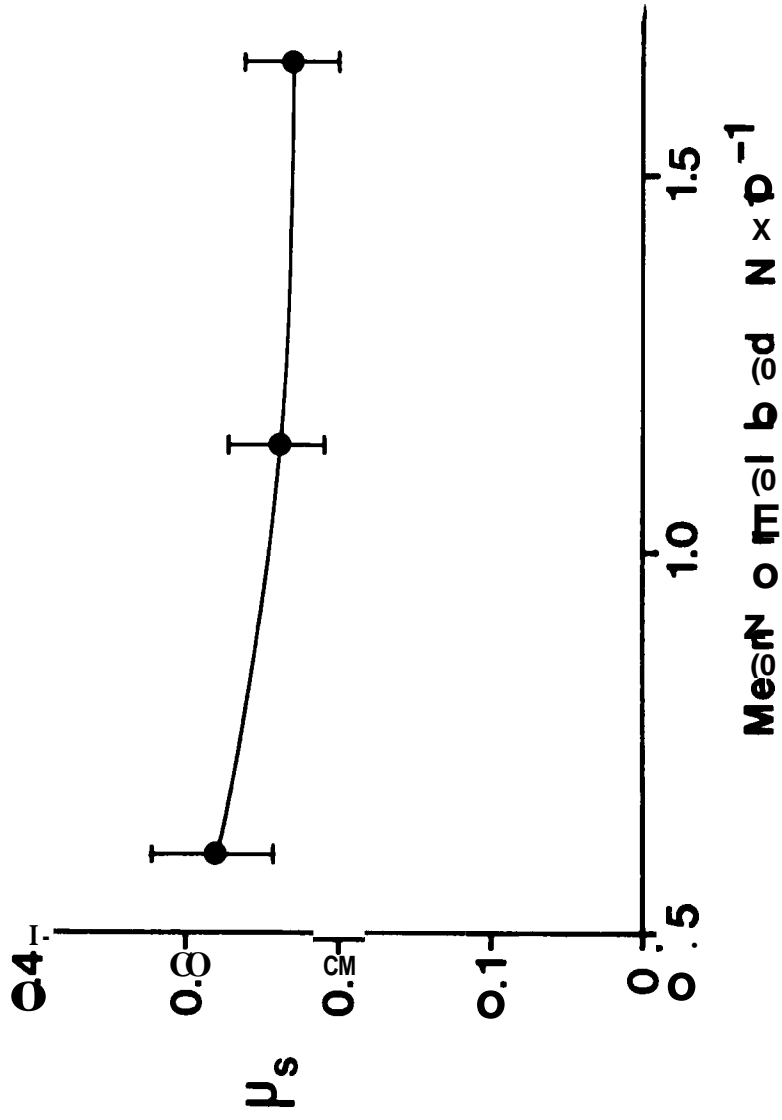


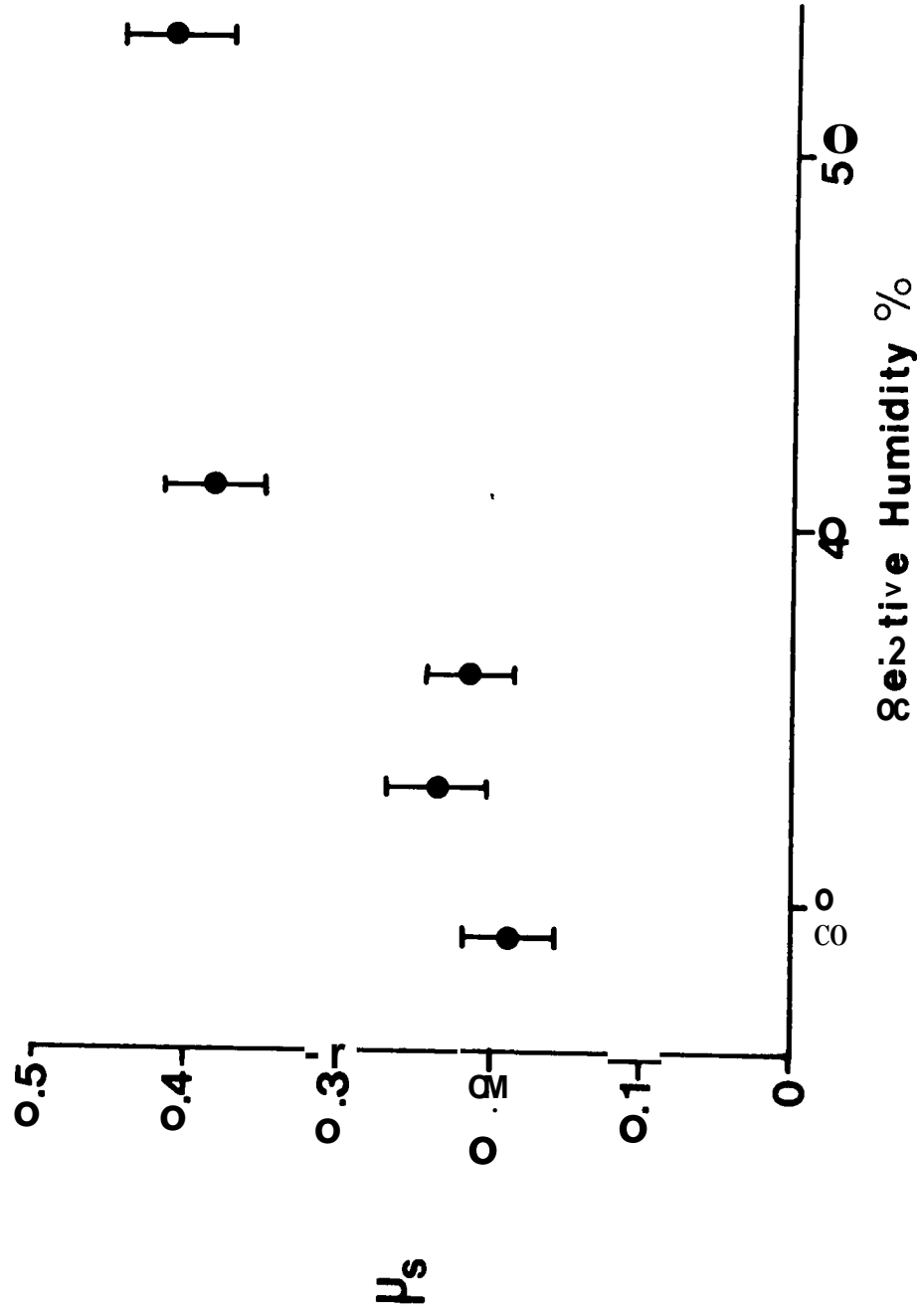
FIGURE 7.4

The Variation of Coefficient of Friction (μ_s) with
Relative Humidity

Gelatin Batch 3

Film conditioned at 20°C for 3 days

Mean \pm 1 std.dev. (n=10)



elevated frictional force. This parallels the increase in γ with temperature observed for many polymers (155). The similarity of results between 29 and 36% R_H suggests that this may be a region where μ_s is minimally affected by humidity. Conditioning and testing humidity was maintained at 33% R_H in subsequent experiments and closer control of R_H at testing than the $\pm 3\%$ provided by the HCTT was achieved by switching off the humidifier, whereupon room conditions remained stable for at least 1 hour.

7.4.4 The Effect of Ageing Time

The ageing of film samples for 3 to 14 days had little effect on the frictional coefficient (Figure 7.5), and the emergence of 'blooming' agents was not apparent.

7.4.5 The Effect of Gelatin Type

Films of each gelatin batch and of a 50 : 50 limed : acid ossein blend were prepared, conditioned and tested together. The results are shown in Table 7,6. Statistical analysis failed to reveal any significant differences between batches and, considering the similarity of their deformational behaviour (Chapter 6), this is not surprising. However, it does show that after 3 days, microconstituents capable of acting as slip additives had not concentrated at the surface of any batch.

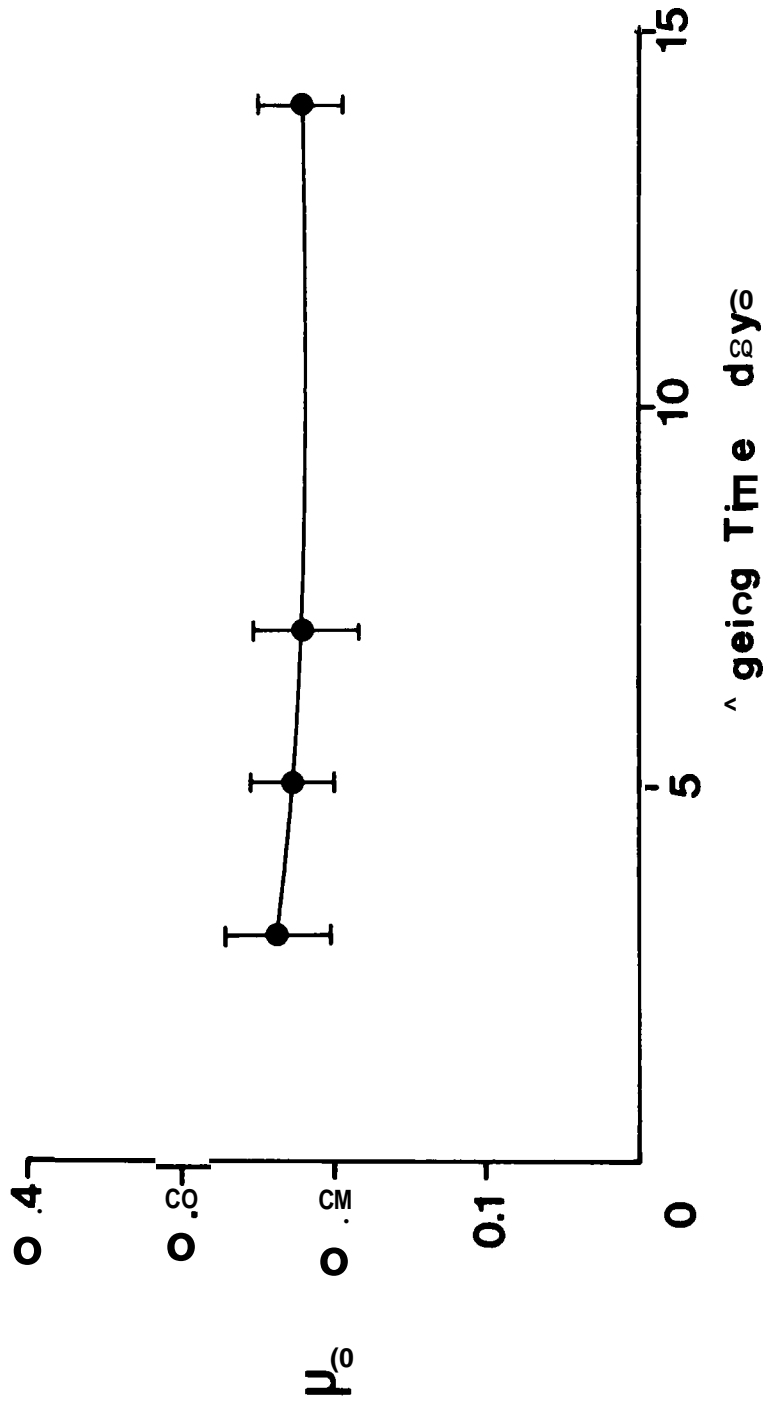
FIGURE 7.5

The Variation of Coefficient of Friction (μ_s) with
Ageing Time

Gelatin Batch 3

Film conditioned at 33% R_H , 20°C

Mean \pm 1 std.dev. (n=10)



Type	Batch	Static Coefficient of Friction (μ_s)	
		Mean	S.D.
Limed ossein (good)	2	0.248	0.033
	3	0.236	0.234
Limed ossein (poor)	4	0.279	0.033
	5	0.256	0.040
	6	0.263	0.040
Acid ossein	7	0.256	0.036
	8	0.260	0.038
Acid pigskin	9	0.299	0.048
50:50 limed/acid ossein blend	2,7	0.276	0.026

Table 7.6

The Variation of Coefficient of Friction with Gelatin Batch

n = 9 to 11

Films conditioned at 33% R_H 20^oC for 3 days

CHAPTER VI I I

CHAPTER VIII

GENERAL DISCUSSION

8.1 DISCUSSION

Only a small number of batches representative of each gelatin type were examined in this work. It must be borne in mind that differences observed between gelatin types may have arisen through fortuitous selection and therefore the conclusions reached on varietal differences between gelatins are tentative.

Each gelatin type exhibited a characteristic shape of molecular mass distribution (**MMD**) derived from the source tissue and treatment undergone during manufacture. Acid ossein gelatins (**AOG**) contained more lower peptides and less intact α chains than limed ossein gelatins (**LOG**). The acid pigskin gelatin (**APG**) contained a larger proportion of high molecular weight species and an intermediate content of lower peptides. This information can be related to film properties.

The overall solution viscosity of a gelatin is directly related to the relative frequency and chain length of the different molecular species present (51). The higher content of low molecular mass (**MM**) species in **AOGs** accounts for their lower viscosities. It has also been suggested that the branched molecules of acid processed gelatins are more compact, by virtue of the remaining crosslinks, than corresponding linear species that occur in **LOGs** (2).

In gelatin gels, molecules of MM 6,000 daltons are considered to remain in the sol phase and not contribute to gel rigidity (51). However, in dry films, on account of the low free volume, low MM species will be entangled in the polymer network and will thereby contribute to mechanical properties. Tensile modulus (TM) is independent of MM in high polymers (198) and as film crystallinity (which does alter TM) did not differ, the tensile moduli of different gelatin films were similar.

Properties involving molecular slippage, such as strain to fracture (FSTN), decrease with decreasing MM (199). This is because short chain molecules have less probability of connecting two or more crystalline regions, and are entangled to a lesser degree thereby reducing the extent of molecular slippage possible before parting company. AOG batches, by virtue of their greater content of lower peptides, would be expected to possess the lowest film FSTN values, and this is the case. However, the **difference** between LOG batches is not satisfactorily explained. LOG batch 2, with a significantly higher FSTN, contains the least lower peptides but less high **MM** species than other LOG batches. It is possible that higher oligomeric molecules may not make a contribution to physical properties consistent with their size, because a proportion of their interactions may be **intramolecular**.

The resolution at the extreme ends of the **MMD** is poor and may not elucidate species responsible for the observed differences in capsules. For example, the microgel particles

and even larger species may act as stress-concentrating nuclei where fracture may occur more readily, thereby weakening the film.

The generation of internal stress during **drying** of the capsule on the moulding pin is of considerable importance. In the film coating of tablets, internal stress may cause an increased incidence of cracking and splitting defects (200); precisely those that arise from the use of 'poor' gelatin batches. Internal stress arises from the dimensional constraint by the metal pin of the cast gel attempting to shrink through loss of solvent volume. In the early stages of drying, free volume, and therefore molecular mobility, is high and internal stress may be dissipated by viscous relaxation involving a greater component of viscous flow. As the film dries, molecular movement is increasingly restricted, viscous relaxation becomes progressively more difficult and internal stress is stored elastically, 'frozen in' to the polymer structure.

Croll (201) has quantified internal stress mathematically;

$$\sigma_1 = \frac{E}{1-\nu} \cdot \frac{\phi_s - \phi_r}{3(1 - \phi_r)}$$

- σ_i : internal stress
- E : tensile modulus
- ν : Poisson's ratio
- ϕ_r : Volume fraction of solvent in the dry film at equilibrium
- ϕ_s : Volume fraction of solvent at the solidification point.

ϕ_s corresponds to the solvent fraction at which the glass transition temperature (T_g) coincides with the temperature of the drying film. In gelatin films this corresponds to a moisture content of around 25% (29).

This equation describes only equilibrium internal stress and assumes thermal contraction of the pin to be negligible. In practice, assuming ideal lubrication, there will be some relief of internal stress by contraction in a direction parallel to the axis of the pin.

If the internal stress exceeds the fracture stress (FSTS) of the **film**, the capsule will crack. However, the stress-strain curve of gelatins under ambient conditions (Fig. 6.7) is such that while FSTS values may remain constant, FSTN values differ. In this case it is the FSTN which determines resistance to internal stress as it denotes the degree to which the material is able to dissipate internal stress by deformation.

Three factors are therefore involved in determining the generation and susceptibility to internal **stress**:

- i) The moisture content at which T_g equates with ambient temperature
- ii) The TM which is directly proportional to internal stress
- iii) The FSTN which determines fracture toughness.

There is evidence that T_g does not differ between limed and acid gelatins at the level of plasticization encountered under equilibrium ambient conditions (29).

Batch 2, a 'good' LOG possessed the lowest TM and a higher FSTN than all other gelatins. It is therefore likely to develop less, and be more resistant to, internal stress. All other gelatins had similar TM values but in terms of FSTN may be grouped as 'intermediate' and 'low' (Fig. 6.14). Those in the 'low' FSTN group, which would be less able to withstand internal stress, were both AOGs and a 'poor' LOG, (batch 6).

Unfortunately no conclusions may be reached concerning the APG batch, because of the variability in FSTN exhibited. In retrospect this was probably caused by a small amount of apparently insoluble filamentous **material** discernable in solution. After blending 5:1 with AOG, all LOG films had statistically indistinguishable FSTN values although the rank order was similar to that for unblended films.

That capsules do suffer internal stress when drying, is apparent from the observation that they shrink after removal from the pin (39). Even if the capsule does not split on the pin, high levels of internal stress will reduce

its ability to withstand externally applied loads, and it will appear to be **more** brittle.

In applying the mechanical testing data to the capsule on the pin two assumptions have been made. Firstly, that the mechanical parameters measured at our necessarily high strain rates are applicable to the rate of strain applied during drying. Secondly, that the film structure is comparable.

The casting conditions were deliberately chosen to match those of capsule formation. However, film properties (except in the drying experiments), have been examined using films dried slowly overnight. This is an imperfect model of the industrial situation where capsules are cast and dried within 40 minutes. Because of the restriction of molecular movement during the later stages of drying, capsules may not attain the degree of helical maturity of slow dried films. The effect of such a decrease in crystallite content would be to shift film properties towards more glassy, brittle behaviour (199). This may explain why few differences between 'good' and 'poor' gelatins were seen in this work whereas they behave differently in practice.

The renaturation rate of individual gelatins might also be important in determining the degree of crystallinity attainable. In addition, the steeper internal moisture content gradient in a rapidly **drying** film would lead to greater internal stress gradients during drying. This effect was observed in this work during attempts to produce flat, rapidly-dried films. The result was twisted and distorted

films that had detached from the glass base.

Overall there was little evidence in this work to suggest reasons for the behaviour of 'poor' LOG batches. 'Poor' batches were indistinguishable in properties from at least one of the two 'good' LOGs in terms of molecular mass distribution, viscosity degradation, foreign protein content, isoionic point, film drying rate, equilibrium moisture content (EMC), degree of helical renaturation and film mechanical properties.

One 'good' LOG appeared to be exceptional in possessing a significantly higher EMC, film fracture strain and α -fraction content. It is possible that the reverse is true; that batch 2 is typical of 'good' LOGs and that batch 3 possessed unusually poor physical properties. Only one piece of evidence supports this; the low specific rotation value of this gelatin which might indicate a lesser degree of helical development. However, the question remains as to why this gelatin performs well during capsule manufacture whilst others do not.

The small but significant differences in EMC between gelatin types have been tentatively ascribed to the degree of exposure of major water binding sites (free carboxyl groups) by deamidation during manufacture (section 5.3.4). An alternate explanation, that the higher EMC is due to higher helical content is not supported by the film specific rotation data.

The higher EMC of batch 2 in comparison with other LOGs may be postulated to be the product of further

deamidation by a prolonged lime pretreatment. Although some 70% of amide groups are deamidated within 8-12 weeks, further liming continues this conversion (71). Prolonged liming would also disrupt greater numbers of inter molecular covalent cross-links facilitating the extraction of the greater numbers of intact α -chain species seen in the MMD of this gelatin. Its low turbidity and pale colour testify to less severe conditions being required for extraction (7).

The films of acid-processed gelatins exhibited significantly higher specific rotation values ($[\alpha]_D^{20}$) than limed ossein films. It is tempting to relate this to a higher helical content brought about by the higher renaturation rates of gelatin molecules in which inter molecular cross-links survive (202). However, the differences were only of the order of 4% of the mean and, as $[\alpha]_D^{20}$ was calculated from independent measurements of rotation, thickness and density, it is questionable whether experimental error was reduced to this extent. In practical terms, polymer film properties are almost certainly insensitive to variations in crystallite content within this range (199). A trivial explanation of the observed variation is a possible decrease in the reliability of polarimeter readings with the higher turbidity of acid gelatins (203).

None of the gelatin batches examined possessed high coefficients of friction. This property is important during the separation and reassembly of capsule shells during filling, and had been suggested responsible for 'brittle' behaviour of capsules. The static frictional test seems a

crude, unreliable and inappropriate test to apply routinely to gelatin as a raw material. For reproducible results, a completely flat, unblemished surface is required; one small protruding bubble or particle affixed to the sliding surfaces gives rise to very large values for the coefficient of friction. In addition, static coefficients do not always correlate with dynamic coefficients of friction; the latter often depending markedly on sliding speed (144).

In addition to the gelatin component, dyes ($\leq 3\%$), titanium dioxide (2%), sodium lauryl sulphate (0.01%) and sodium **metabisulphate** are regularly present in the capsule shell. The influence of these additives on film formation and physical properties would merit further investigation. Many FD & C dyes interact with gelatins in solution, and anionic dyes have been shown to bind to a greater extent to the more cationic acid-pretreated gelatins (204,205) and to a degree **concomitant** with the polarity of the dye (206). As ionic groups in gelatin are major centres for water binding, a competing association at these sites could decrease water sorptive capacity, although hydration of the dye molecules themselves, and their large size, would reduce this effect compared to that observed after the addition of, for example, urea (207).

In addition to ionic binding there is also evidence to suggest the involvement of hydrophobic and other types of binding (204,208). Dye competition at **polymer:polymer** binding sites (e.g. the hydrogen bonding that maintains helical conformati^o_An) could alter gelatin renaturation,

structure and subsequent physical properties. Artemova et al (7) have observed partial inhibition of structure formation by 0.02% w/w dyes in 3-6% gels whilst others (8) report the formation of highly elastic or brittle gels on addition of a colour component. Although in mature films changes in the degree of hydrogen binding have only been detected at high dye concentrations (10%)(204), Kellaway et al (205) have observed a reduction in strain-induced contraction inversely related to erythrosine concentration within the range 0.2-2% w/w added dye. These authors also concluded that the incorporation of 2% titanium dioxide had little effect on film tensile modulus. However, experience with other polymers suggests that the addition of particulate solids increases the cracking and splitting defect incidence by increasing the tensile modulus and lowering fracture stress (200).

8.3 SUGGESTIONS FOR FURTHER WORK

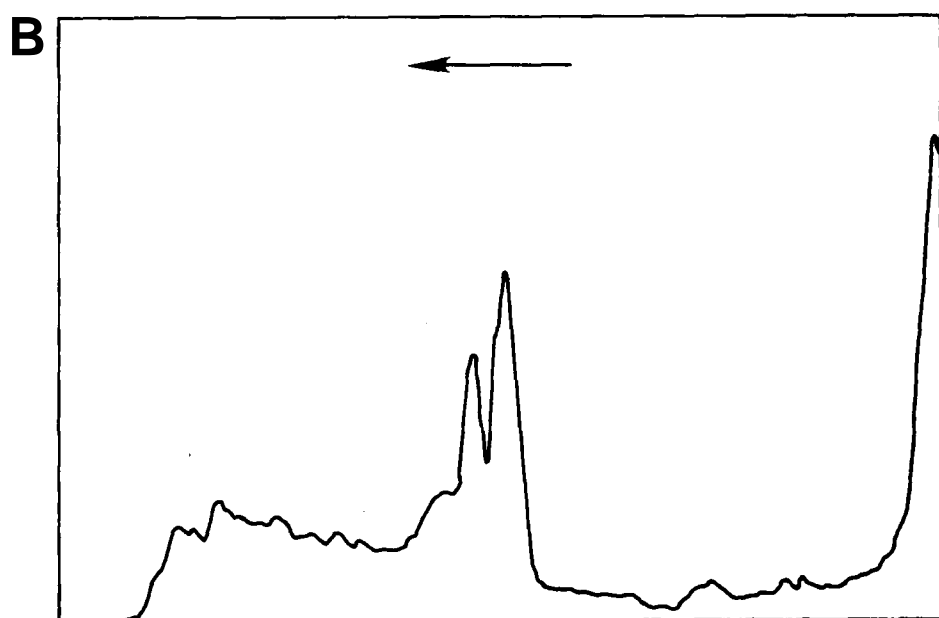
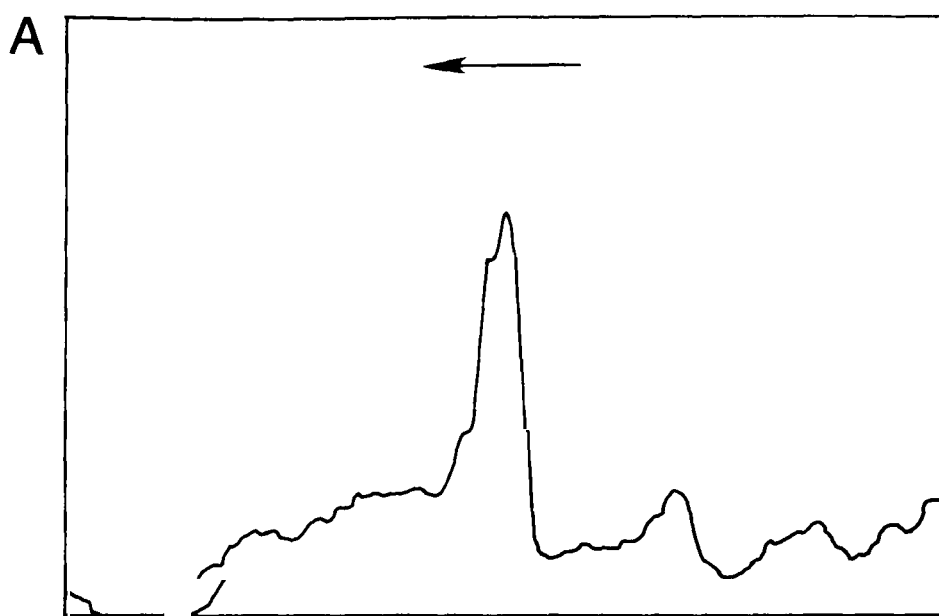
The author considers the following worthy of further investigation with respect to varietal differences between gelatin films and '**good**' and '**poor**' batches for hard capsule production.

- A) The effect of the rapidity of **drying** on film helical maturity and mechanical properties.
- B) The development of internal stress in restrained wet films subject to different drying conditions.

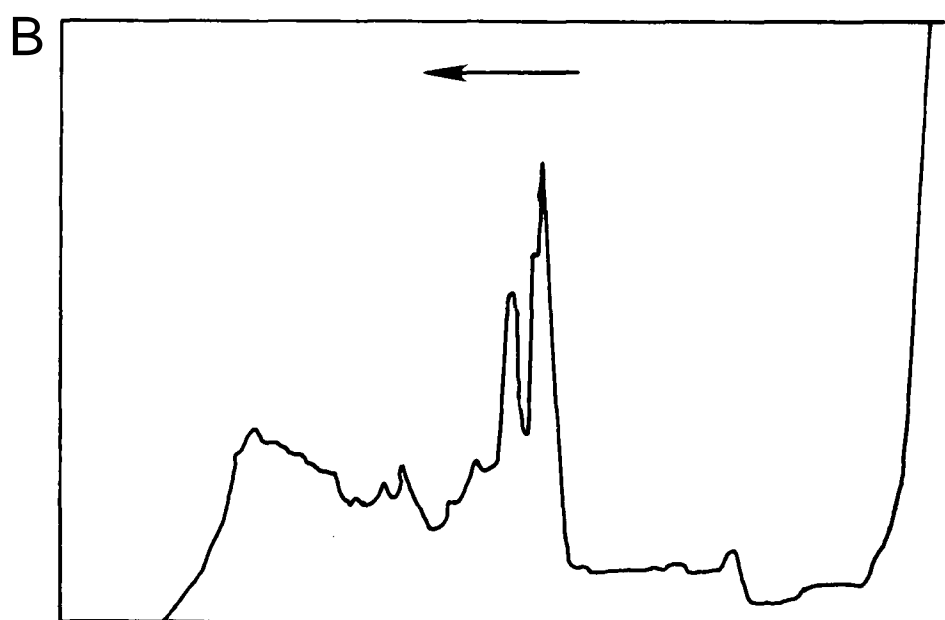
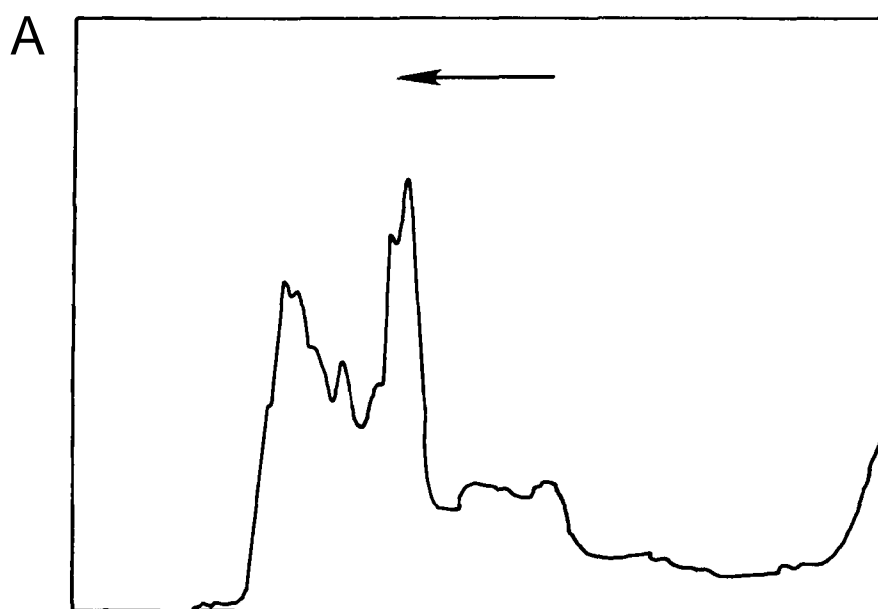
- C) The effect of dyes and other excipients on structural development, mechanical properties, the glass transition temperature, drying rates, equilibrium moisture content and the generation of internal stress.
- D) An extension of the work to a greater number of gelatin batches in those properties (such as equilibrium moisture content) where this work suggests varietal differences.

APPENDIX 1

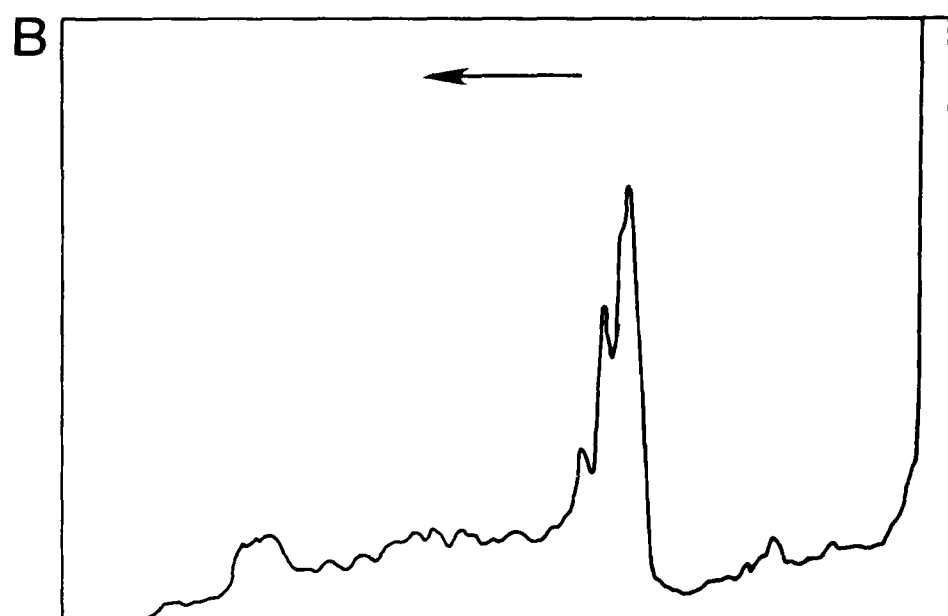
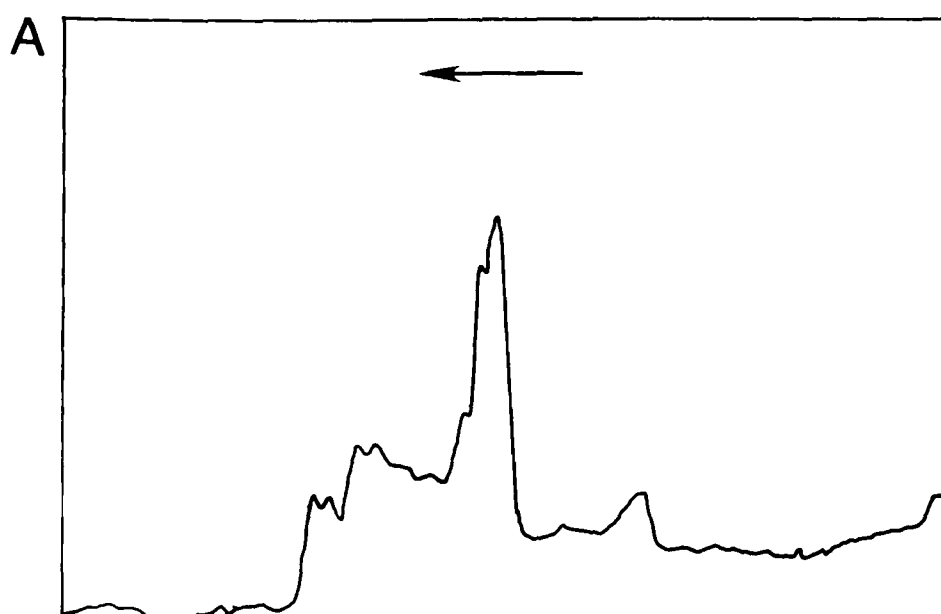
Densitograms of electrophoretic separations of the remaining gelatin batches. Trace A was performed on a 2.5% gel rod, trace B on a 5% rod. LOG is limed ossein gelatin, AOG, acid ossein gelatin.



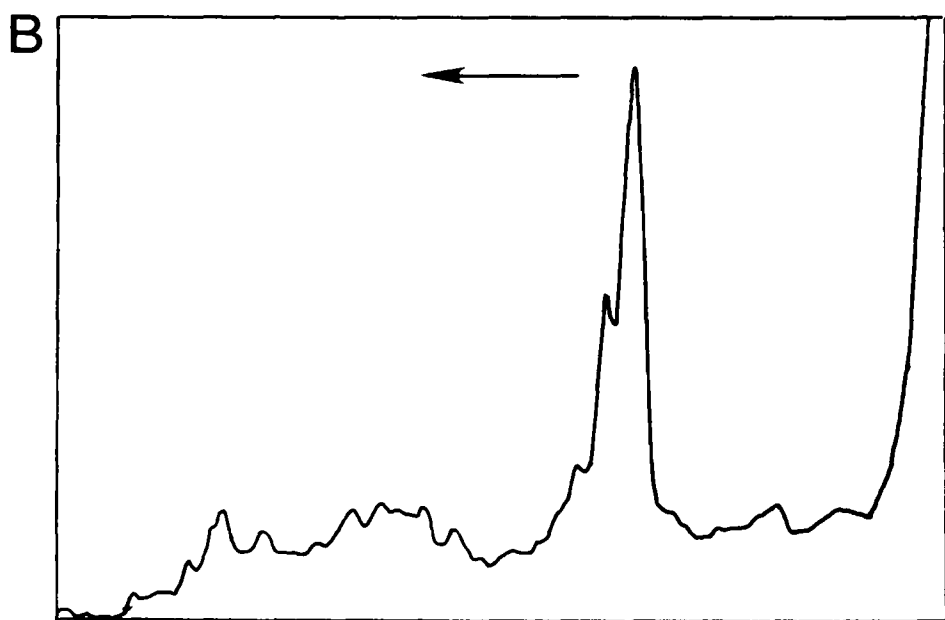
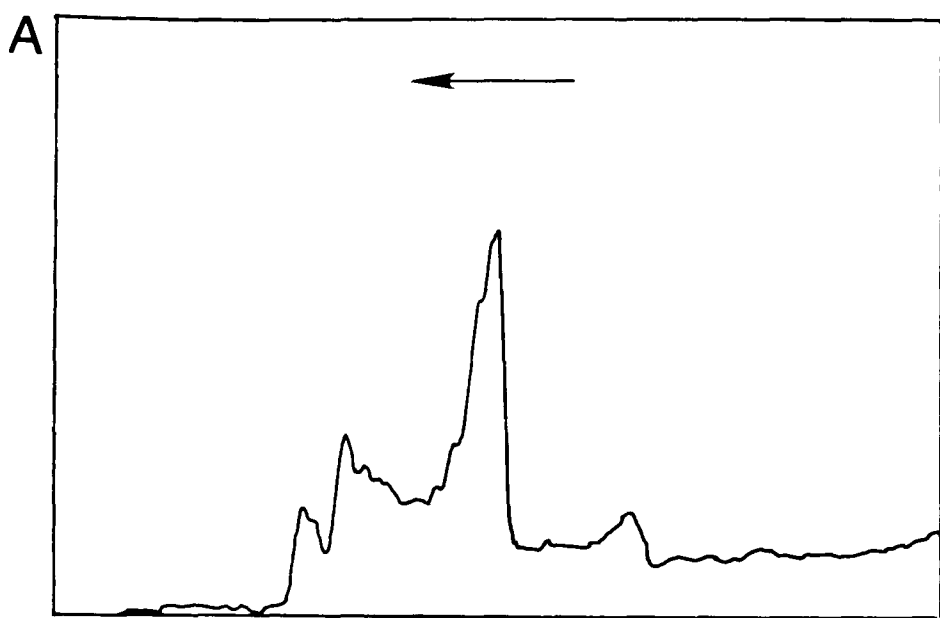
tOG Batch 2



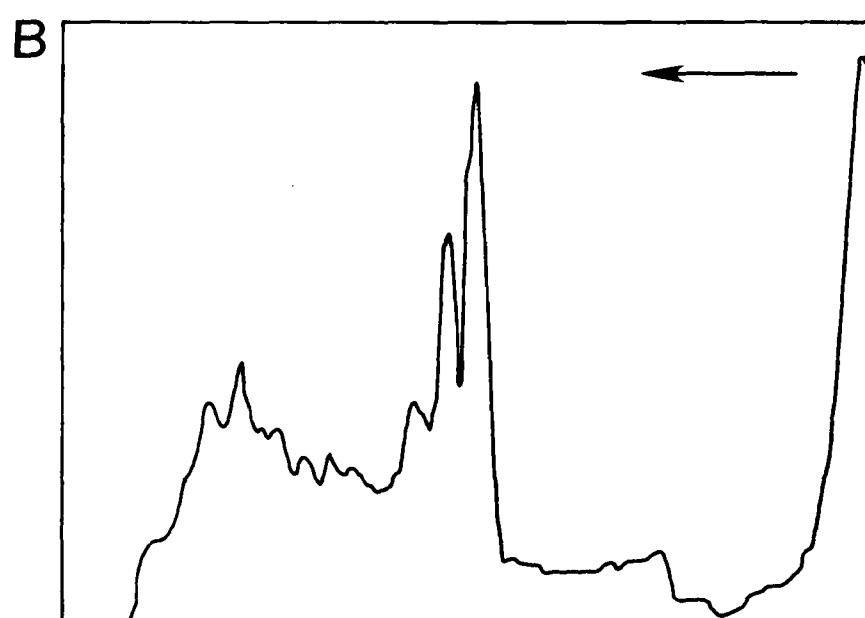
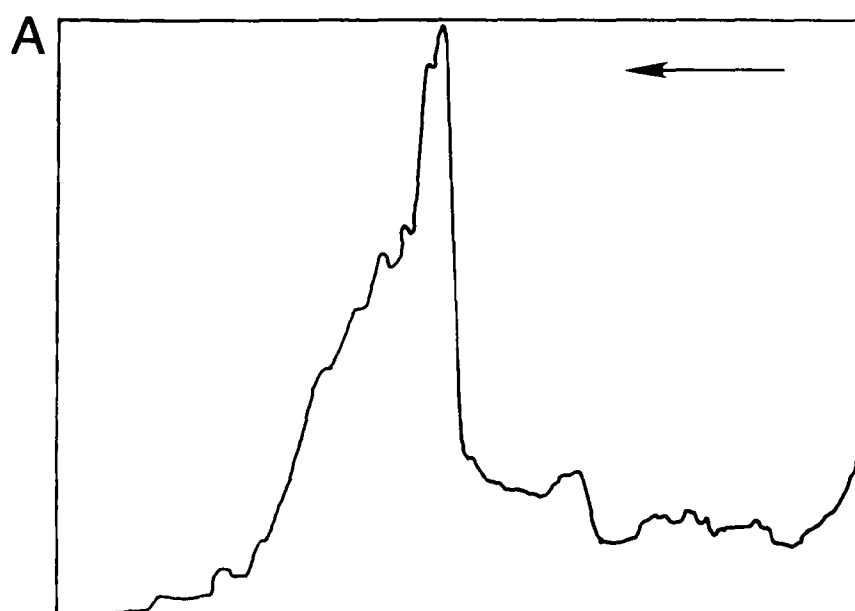
LOG Batch 3



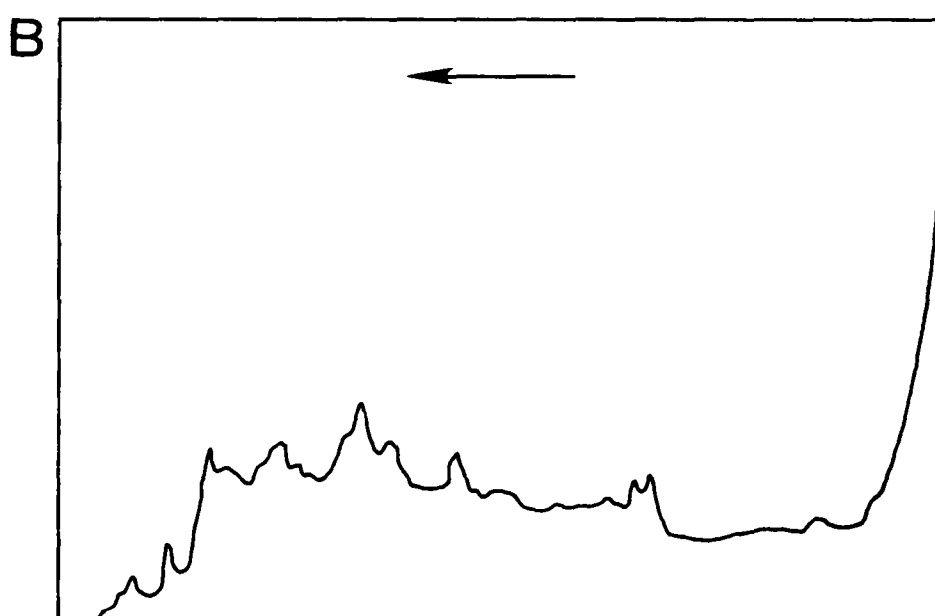
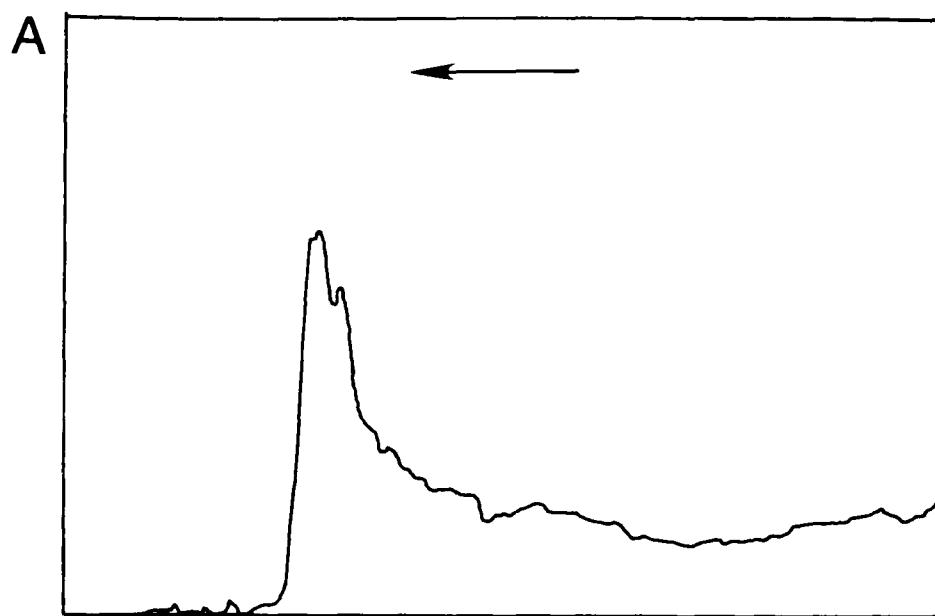
LOG Batch 4



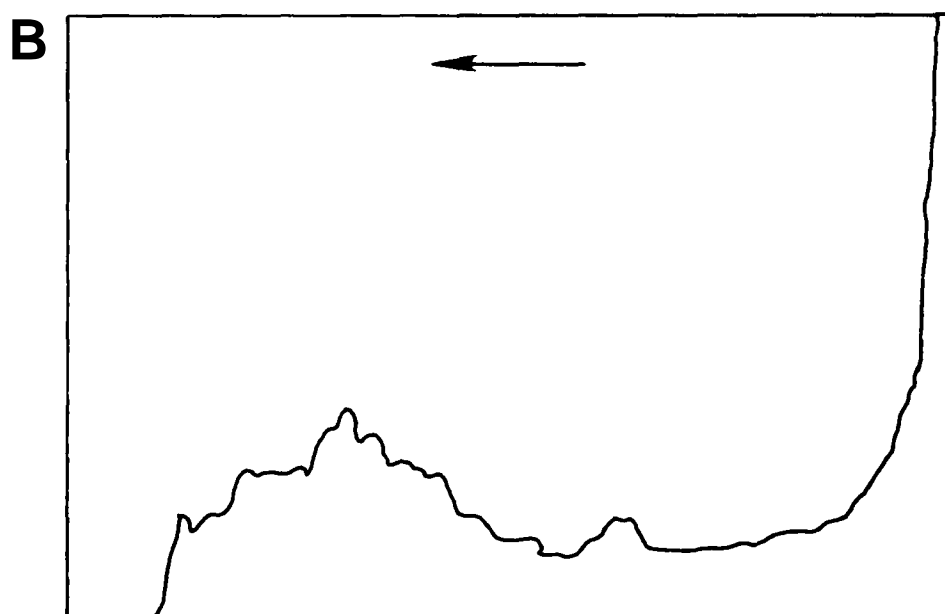
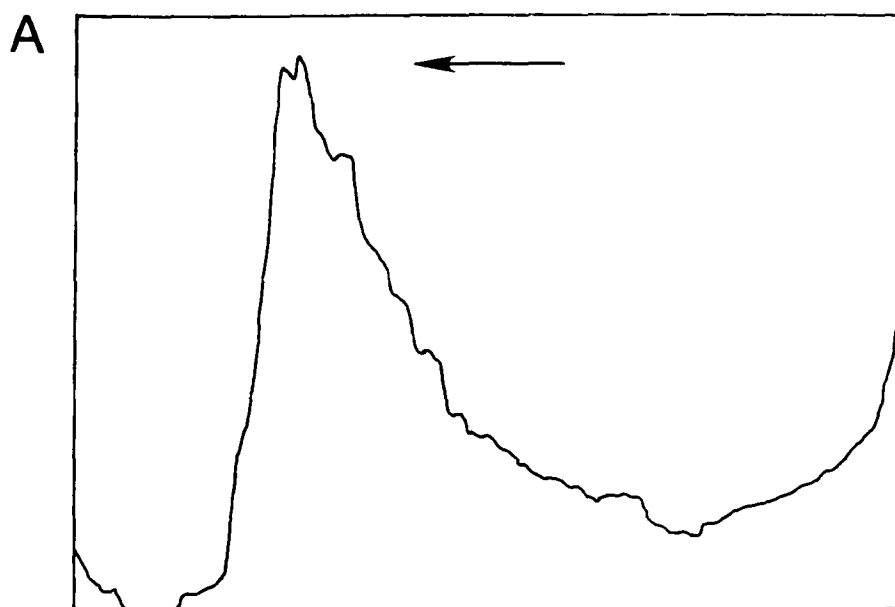
LOG Batch 5



LOG Batch 6



AOG Batch 7



AOG Batch 8

APPENDIX 2

The Fortran program used for the calculation of drying rates from **weight/time** drying curves. This program fits cubic equations to segments of the drying curve and drying rates are calculated by taking derivatives of these equations. The results are both tabulated, and presented as graphs of drying rate against time and rate against percentage mean moisture content.

```

LIST
LIBRARY(ED,L(1))
LIBRARY(ED,L(2))
LIBRARY(ED,L(3))
LIBRARY(ED,L(4))
LIBRARY(ED,L(5))
LIBRARY(ED,L(6))
PROGRAM(GENF)
EXTENDED
INPLI5,20=CR0
OUTPUT6,30=LP0
OUTPUT 106=LP14
CREATE 107=MT14/FORHATTED(A)/256
TRACE2
COMPRESS INTEGER AND LOGICAL
END

```

```

MASTER FRED
DIMENSION X(300),Y(300),U(300),D(300),XP(300),YP(300),
1      XK(50),W2(3,50),C(50),PER(300)
READ(5,201) SOLID,GBAL
READ(5,200) N
IF (N.GT.300) CALL EXIT
WRITE(6,102)
DO 10 I=1,N
U(I) = 1.0
READ(5,201) X(I),Y(I)
WRITE(6,103) X(I),Y(I)
10  CONTINUE
C
C  SET UP KNOTS ETC.
C
      XSTEP = (X(N)-X(1))/5
      NCAP5 = 5
      NCAP7 = NCAP+7
      NCAP3 = NCAP+3
      DO 15 I=5,NCAP3
15  XK(I) = XSTEP*FLOAT(I-4)+1
      CONTINUE
      IFAIL = 0
      CALL EC2DAF(N,NCAP7,X,Y,W,XK,D,W2,C,SS,IFAIL)
      WRITE(6,104) SS

```

```

C
C NOW CALCULATE DERIVATIVES
C
WRITE(C,101)
XSTEP = (X(N)-X(1))/299.0
DO 20 I=1,300
XX = FLOAT(I-1)*XSTEP*X(1)
IF (XX.GT.X(N)) GO TO 25
IFAIL = 0
CALL SPDERIV(NCAP7,XK,C,XX,RESS,IFAIL)
XP(I) = XX
D(I) = RESS
20 CONTINUE
25 CONTINUE
DO 30 I=1,300
XX = XP(I)
IF (XX.GT.X(N)) GO TO 35
NP = I
IFAIL = 0
CALL ED2BBF(NCAP7,XK,C,XX,YPP,IFAIL)
YP(I) = YPP
C
C NOW CALCULATE PERCENTAGE LIQUID
C
WTOT = YPP+CBAL
PER(I) = (WTOT-SOLID)/WTOT*100.0
30 CONTINUE
WRITE(C,100)(XP(I),D(I),PER(I),I=1,300)
35 CONTINUE
C
C PLOT GRAPHS
C
CALL GINBEG(10,0)
CALL GRAF(XP,YP,NP,0)
CALL GRASYN(X,Y,N,B,0)
CAU PICCLE
CALL GRAF(XP,D,NP,0)
CALL PICCLE
CALL GRAF(PER,D,NP,0)
CALL GINFIN
100 FORMAT(1H ,F7.3,2E20,10)
101 FORMAT(1H1,' XX DERIV
1 1 ----
102 FORMAT(1H1,' DATA/
1 1 -----/)
103 FORTC1H ,2F12.5)
104 FORMAT(1H ,2E20,10//)
200 FORMAT(10)
201 FORMAT(2F0.0)
STOP.....
END

```

LENGTH 416, NAME FRED

SUBROUTINE SPDERIV(NCAP7,K,C,X,SD,IFAIL)

REAL K

DIMENSION K(NCAP7),C(NCAP7)

REAL K1,K2,K3,K4,K5,K6,K4K2,K5K3,K5K2

DOUBLE PRECISION SRNAME

DATA SRNAME /8HSPDERIV /

RETURNS IN SD THE VALUE AT X OF THE DERIVATIVE
OF THE P-SPLINE DEFINEDS BY K AND C

NCAP = NCAP7-7

IF ((X.GE.K(4)).AND.(X.LE.K(NCAP+4))) GO TO 10

IERROK = 1

CALL F01AAF(IFAIL,IERROK,SRNAME)

RETURN

CONTINUE

FIND INTERVAL

II = 4

CONTINUE

IF (X.LE.K(II+1)) GO TO 20

II = II+1

GO TO 15

CONTINUE

J = II+1

NN = ICAP+4

J1=J-1

K1FK(J1-2)

K2FK(J1-1)

K3FK(J1)

K4FK(J)

K5FK(J+1)

K6FK(J+2)

CJFC(J+4)

C2FC(J+3)

C3FC(J+2)

CJ3FC(J+1)

K4K2=K4-K2

K5K3=K5-K3

K5K2=K5-K2

ALP = 1.0/(K4-K3)

ALP1=ALP/((K5K3)*(K6-K3))

ALP2=ALP/((K4K2)*(K4-K1))

ALP3=ALP/((K5K3)*(K5K2))

ALP4=ALP/((K4K2)*(K5K2))

CX3 = ALP1*(CJ3-C3) + ALP2*(C2-CJ)*

```

1      (ALP3+ALP4)*(C2+C3)
CX2 = ALP1*(-3*K3*CJ3 + K6*C3 + 2*K3*C3) +
1      ALP2*(-(K4*K4 + K1)*C2 + 3*K4*CJ) +
2      ALP3*( (K5+K3+K2)*C3 - (K5+K5+K3)*C2) +
3      ALP4*( (K4+K2+K2)*C3 - (K5+K4+K2)*C2)
CX1 = ALP1*( 3*K3*K3*CJ3 - ((K6+K6+K3)*K3)*C3) +
1      ALP2*( (K4*(K4+K1+K1))*C2 - 3*K4*K4*CJ) +
2      ALP3*( -(K3*K5+K2*(K5+K3))*C3 + (K5*(K5+K3+K3))*C2) +
3      ALP4*( -(K2*(K2+K4+K4))*C3 + ((K4+K2)*K5+K2*K4)*C2)
CX0 = ALP1*( -K3*CJ3 + K6*C3)*K3*K3 +
1      ALP2*( -K1*C2 + K4*CJ)*K4*K4 +
2      ALP3*( K2*C3 - K5*C2)*K3*K5 +
3      ALP4*( K2*C3 - K5*C2)*K2*K4

```

C
C
C

CALCULATE DERIVATIVE

SD = (3.0*CX3*X+2.0*CX2)*X*CX1

RETURN

END

APPENDIX 3

THE STATISTICAL ANALYSIS OF EXPERIMENTAL RESULTS

A) Homogeneity of Variance

Homogeneity of variance **between** groups of data for comparison was analysed by means of the F-statistic (taken as the **maximum/minimum** variance ratio) at a significance level of $p = 0.05$.

$$F = \frac{(S_1)^2}{(S_2)^2}$$

F : maximum/minimum variance ratio

S_1, S_2 : standard deviation of the mean of group
of groups 1 and 2

B) Significance Testing between Groups having Homogenous Variances

Data **comprising** 7 or less groups were compared by paired two-tailed student t-tests.

$$t = \frac{m_1 - m_2}{\sqrt{1/n_1 + 1/n_2}}$$

t : t statistic

m_1, m_2 : mean of groups 1 and 2

S_1, S_2 : standard deviation of groups 1 and 2

n_1, n_2 : number of observations in groups 1 and 2

where

$$\sigma = \frac{n_1(S_1)^2 + n_2(S_2)^2}{n_1 + n_2 - 2}$$

σ : best estimate of population standard deviation

t was compared with tabulated values at degrees of freedom = $n_1 + n_2 - 2$. (211).

If applied to 7 or more groups these tests involve more than 20 individual comparisons and at a significance level of $p = 0.05$ thereby may include false significant results. Data comprising 7 or more groups were compared by means of the Scheffe Range Test. This test calculates an F-statistic for each paired comparison and the result is compared with a critical value of F calculated from a one-way analysis of variance (ANOVA).

$$F_s = \frac{(M_1 - M_2)^2}{M_s(n_1+n_2)/n_1 \cdot n_2}$$

- F_s : Scheffe F-statistic
- M_s : mean square value used as denominator in ANOVA
- k : number of groups
- F : critical value at the desired level of significance from ANOVA

F_s is significant if it exceeds $F(k-1)$.

C) Significance Testing between Groups with Non-Homogenous Variances

These groups were compared by paired, two-tailed Mann-Whitney U-Tests. In this test the observations of the two groups for comparison are ranked and a sum of rank values calculated. The U statistic may then be calculated for each group;

$$U_1 = n_1 n_2 + \frac{n_1 (n_1 + 1)}{2} - \Sigma R_1$$

U_1 : U value of group 1

ΣR_1 : Sum of ranks for group 1.

The smaller of the two **U-values** is compared with tabulated values (211) to indicate the level of significance.

D) Linear Correlation

Linear correlation between two independent variables was tested using Pearsons product moment correlation coefficient (r).

$$r = \frac{N \Sigma xy - \Sigma x (\Sigma y)}{(N \Sigma x^2 - (\Sigma x)^2) \cdot (N \Sigma y^2 - (\Sigma y)^2)}$$

N : number of pairs of observations

x,y : individual observations of groups x and y

A level of significance may be deduced for r by reference to tabulated data (212).

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